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Fluorinated and iodinated templates for syntheses of β-turn peptidomimetics

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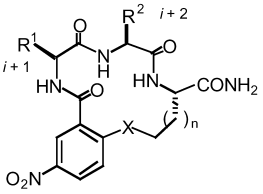
Abstract—Various 2-fluoro-5-nitrobenzoic acids and the homocysteine derivative **2** have been combined in solid phase syntheses of the peptidomimetic types **3**–**6**. NMR and CD data collected for some of these compounds indicate that a transannular S*O* to *H*N hydrogen bond stabilizes β-turn conformations for the sulfones and one of the sulfoxide epimers. An extensive library of compounds was made and studied to test this assertion.

1. Introduction

Fluoronitrobenzoic acid derivatives are useful electrophiles for nucleophilic aromatic substitution reactions (S_NAr), and they have been used in diverse solid phase syntheses for various purposes.[1–5] In these labs, such substrates have been used in solid phase macrocyclization reactions to form β-turn mimics of the type **A**.[6–10] We are interested in using such compounds to mimic or disrupt protein–protein interactions, especially those between the nerve growth factor (NGF) and its high affinity receptor (TrkA).[11] To that end, many compounds have been prepared in this series, mostly with X=N or O.



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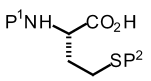

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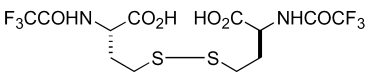

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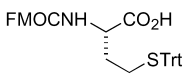
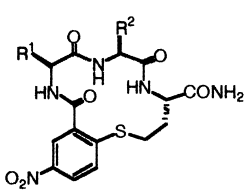


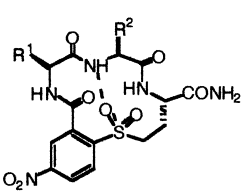
2

Until recently, a conspicuous omission in the studies of turn analogs **A** has been the compounds where X=S and n=1, i.e. homocysteine derivatives. They were not investigated extensively due to the cost and difficulties associated with obtaining protected, enantiomerically pure, homocysteine starting materials, **B**.[12–16] The work described here was made possible by a gift of 100 g of the homocysteine derivative **1** from Bristol Myers Squibb. In preliminary work, now communicated, we transformed this into an appropriately protected derivative **2**, and made a small library of compounds **A** (X=S, SO {2 epimers}, and SO_2; n=1). It was established that the cyclic thiols **A** did not show any pronounced conformational bias towards β-turn conformations in DMSO solution. Curiously, the corresponding sulfones (X=SO_2), and one of the sulfoxide epimers (X=SO) did have biases towards β-turn conformations in solution.[17]

The observations described above led us to propose that the β-turn conformations could be stabilized by transannular S*O* to i+2N*H* hydrogen bonds, as illustrated in Fig. 1. We also proposed that the absolute stereochemistry of the sulfoxide chiral centers could be predicted via conformational analyses. While stereochemistries assigned in this way are not incontrovertible, the weight of evidence in their favor is substantial, and becomes more compelling as the number of compounds studied is increased. The preliminary study focused on a series of 14 compounds, but here a more extensive study is reported.



sulfide: not a β-turn (no transannular H-bond)



sulfone: β-turn observed (transannular H-bond possible)

Figure 1. β-Turn conformations and S*O* to *H*N transannular H-bonds may be intimately linked.

Keywords: β-turn peptidomimetics; fluoronitrobenzoic acid derivatives; nerve growth factor.

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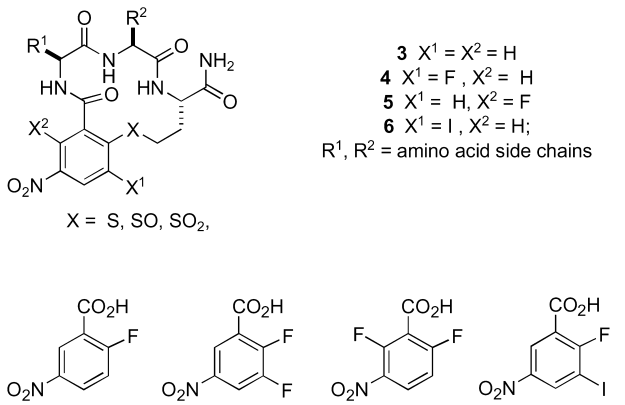
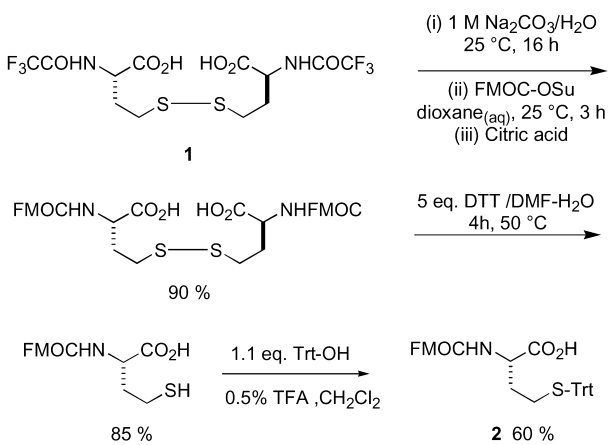
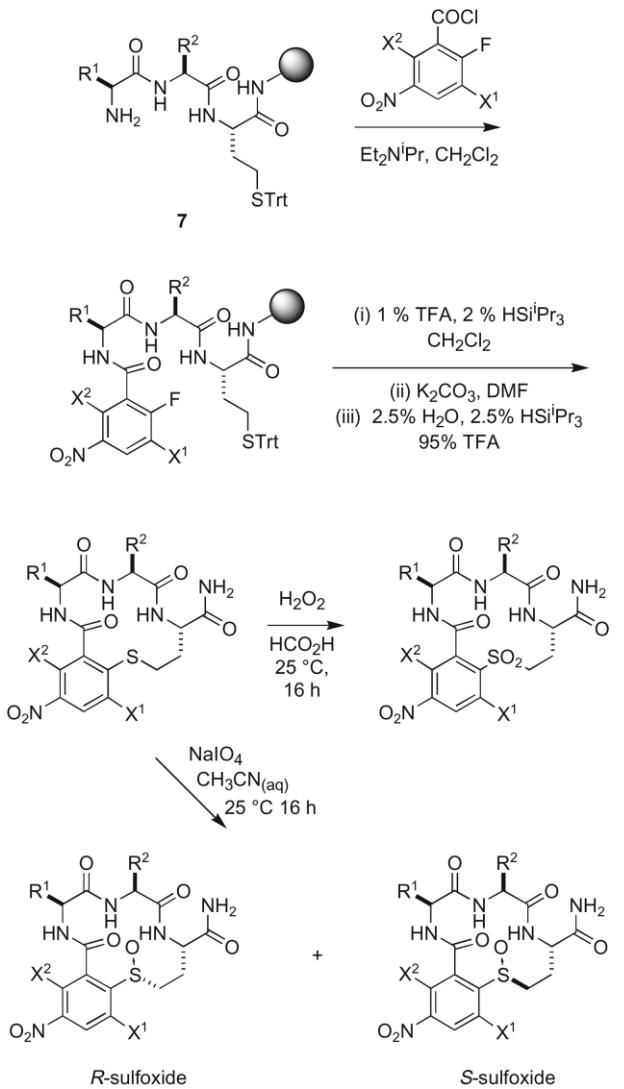


Figure 2. Fluoronitrobenzoic acid derivatives used to prepare the peptidomimetics.

This paper describes work designed to test if β-turn conformations could be correlated with the sulfones and one configuration of the sulfoxide stereoisomer for a much larger set of compounds. Thus solid phase syntheses of a library of 60 peptidomimetics **3**–**6** is described. To do this, other electrophilic templates for the S_NAr reaction were used in addition to 2-fluoro-5-nitrobenzoic acid, the template used in the work communicated (giving some of the compounds **3** in Fig. 2). Specifically the difluorinated and iodinated templates also shown in Fig. 2 were used; these were chosen on the basis of commercial availability or ease of preparation. The oxidation state of the sulfur was also varied. Conformational analyses (various NMR experiments and CD studies) have now been performed for a total of 20 compounds. These experiments were designed to reveal if β-turn conformations were always observed for the sulfone and one stereoisomer of the sulfoxides. For nine compounds, these physical data were then compared with computer simulated (via quenched molecular dynamics (QMD))[18,19] conformers to identify ones that are predicted to be highly populated, low in energy, and match the NMR data well. Those experiments facilitate correlations of the NMR data with the predicted favorable conformations, to further gauge the degree of confidence that can be ascribed to configurational assignments in this series.



Scheme 1. Synthesis of **2**.



Scheme 2. Synthesis of the peptidomimetics **3**–**6**.

2. Syntheses of peptidomimetics 3–6

Scheme 1 illustrates how the disulfide **1** was transformed into the protected amino acid **2**. Briefly, the trifluoroacetate groups of **1** were removed under basic conditions, the amine functionalities were FMOC-protected, then the disulfide was reduced and the free thiol group was protected with a trityl group.

Preparation of the cyclic macrocycles **3**–**6** (Scheme 2) began with a series of HBTU/HOBt[20] couplings following a conventional FMOC approach[21] to obtain the open chain intermediates **7**, which were then capped with the appropriate fluoronitrobenzoic acid derivative (as indicated in Fig. 2). The *S*-Trt group was then removed with dilute trifluoroacetic acid allowing the S_NAr macrocyclization to proceed. Cyclic thioethers of the series **3**–**6** were obtained at that stage by cleavage from the resin. Subsequently, oxidations to the sulfone and sulfoxide compounds were performed in solution. Thus, the sulfones were prepared using hydrogen peroxide in formic acid, while the sulfoxides were formed via treatment with sodium periodate in aqueous acetonitrile.

Table 1. Peptidomimetics **3**–**6** prepared as indicated in Scheme 2

Compound	X	X^1	X^2	R^1	R^2	Crude HPLC purity (%)[a]	Isolated yield (%)
3a	S	H	H	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	87	32
3b	S	H	H	H	$(CH_2)_4NH_2$	88	34
3c	S	H	H	$CH(CH_3)C_2H_5$	$(CH_2)_4NH_2$	84	31
3d	S	H	H	$CH(CH_3)C_2H_5$	$(CH_2)_3NHC(=NH)NH_2$	84	27
3e	S	H	H	CH_2CONH_2	CH_2CONH_2	81	22
3f	S	H	H	$CH_2C_6H_4OH$	H	90	38
3g	S	H	H	CH_3	CH_3	90	34
3h	S	H	H	$CH_2C_6H_5$	$CH_2C_6H_4OH$	93	37
3i	S	H	H	$CH(CH_3)C_2H_5$	$CH_2C_6H_5$	95	42
3j	(*R*)-SO	H	H	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	30	5
3k	(*S*)-SO	H	H	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	62	8
3l	(*R*)-SO	H	H	$CH_2C_6H_5$	$CH_2C_6H_4OH$	18	4
3m	(*S*)-SO	H	H	$CH_2C_6H_5$	$CH_2C_6H_4OH$	70	16
3n	(*R*)-SO	H	H	$CH(CH_3)C_2H_5$	$CH_2C_6H_5$	22	4
3o	(*S*)-SO	H	H	$CH(CH_3)C_2H_5$	$CH_2C_6H_5$	68	13
3p	SO_2	H	H	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	81	21
3q	SO_2	H	H	H	$(CH_2)_4NH_2$	82	26
3r	SO_2	H	H	$CH(CH_3)C_2H_5$	$(CH_2)_4NH_2$	75	27
3s	SO_2	H	H	$CH(CH_3)C_2H_5$	$(CH_2)_3NHC(=NH)NH_2$	78	19
3t	SO_2	H	H	CH_2CONH_2	CH_2CONH_2	71	16
3u	SO_2	H	H	$CH_2C_6H_4OH$	H	85	31
3v	SO_2	H	H	CH_3	CH_3	85	28
3w	SO_2	H	H	$CH_2C_6H_5$	$CH_2C_6H_4OH$	87	32
3x	SO_2	H	H	$CH(CH_3)C_2H_5$	$CH_2C_6H_5$	83	27
4a	S	F	H	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	81	33
4b	S	F	H	H	$(CH_2)_4NH_2$	42	12
4c	S	F	H	$CH(CH_3)C_2H_5$	$(CH_2)_4NH_2$	65	18
4d	S	F	H	$CH(CH_3)C_2H_5$	$(CH_2)_3NHC(=NH)NH_2$	85	27
4e	S	F	H	CH_2CONH_2	CH_2CONH_2	87	21
4f	S	F	H	$CH_2C_6H_4OH$	H	79	24
4g	SO_2	F	H	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	79	25
4h	SO_2	F	H	H	$(CH_2)_4NH_2$	32	5
4i	SO_2	F	H	$CH(CH_3)C_2H_5$	$(CH_2)_4NH_2$	46	12
4j	SO_2	F	H	$CH(CH_3)C_2H_5$	$(CH_2)_3NHC(=NH)NH_2$	78	17
4k	SO_2	F	H	CH_2CONH_2	CH_2CONH_2	81	21
4l	SO_2	F	H	$CH(OH)CH_3$	H	72	20
5a	S	H	F	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	85	32
5b	S	H	F	H	$(CH_2)_4NH_2$	72	19
5c	S	H	F	$CH(CH_3)C_2H_5$	$(CH_2)_4NH_2$	81	27
5d	S	H	F	$CH(CH_3)C_2H_5$	$(CH_2)_3NHC(=NH)NH_2$	92	42
5e	S	H	F	CH_2CONH_2	CH_2CONH_2	85	35
5f	S	H	F	$CH_2C_6H_4OH$	H	79	22
5g	SO_2	H	F	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	79	26
5h	SO_2	H	F	H	$(CH_2)_4NH_2$	65	11
5i	SO_2	H	F	$CH(CH_3)C_2H_5$	$(CH_2)_4NH_2$	70	25
5j	SO_2	H	F	$CH(CH_3)C_2H_5$	$(CH_2)_3NHC(=NH)NH_2$	85	31
5k	SO_2	H	F	CH_2CONH_2	CH_2CONH_2	82	22
5l	SO_2	H	F	$CH_2C_6H_4OH$	H	70	14
6a	S	I	H	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	88	28
6b	S	I	H	H	$(CH_2)_4NH_2$	77	18
6c	S	I	H	$CH(CH_3)C_2H_5$	$(CH_2)_4NH_2$	54	12
6d	S	I	H	$CH(CH_3)C_2H_5$	$(CH_2)_3NHC(=NH)NH_2$	85	32
6e	S	I	H	CH_2CONH_2	CH_2CONH_2	91	35
6f	S	I	H	$CH_2C_6H_4OH$	H	86	28
6g	SO_2	I	H	$(CH_2)_2CO_2H$	$(CH_2)_4NH_2$	84	22
6h	SO_2	I	H	H	$(CH_2)_4NH_2$	71	18
6i	SO_2	I	H	$CH(CH_3)C_2H_5$	$(CH_2)_4NH_2$	34	6
6j	SO_2	I	H	$CH(CH_3)C_2H_5$	$(CH_2)_3NHC(=NH)NH_2$	77	28
6k	SO_2	I	H	CH_2CONH_2	CH_2CONH_2	87	28
6l	SO_2	I	H	$CH(OH)CH_3$	H	81	23

[a] As assessed by UV detection at 254 nm.

Table 1 shows the compounds that were prepared, the HPLC purities of the crude materials, and the isolated yields. Most of the compounds shown in Table 1 are sulfides and sulfones. Considerably fewer sulfoxides were prepared since these formed as a mixture of epimers that were not always separable by HPLC. The possibility that macrocyclization products **5** were formed via displacement of the fluoride *ortho* to the nitro was easily excluded by observing the large $^3J_{FH}$ coupling constants for the remaining fluorine atom.

Table 2. Key NMR data for some sulfide peptidomimetics in the series **3**–**6**

Parameter[a]	Sulfides							Ideal β-I turn
	3a	**3g**	**3h**	**3i**	**4a**	**5a**	**6a**	
NH_{i+2} T_c (−ppb/K)	0.0	0.0	1.6	1.2	4.8	5.1	5.3	N/A
NH_{i+2} H/D exchange	Fast	Slow	Slow	Fast	Fast	Medium	Slow	N/A
NH_{i+3} T_c (−ppb/K)	6.37	6.6	6.0	6.0	3.25	3.5	3.5	<3.0
NH_{i+3} H/D exchange	Medium	Slow	Slow	Fast	Slow	Slow	Slow	Slow
^3JC$_\alpha$-NH$_{i+1}$ (Hz)	8.5	8.0	8.5	9.0	6.0	6.5	6.0	4.0
^3JC$_\alpha$-NH$_{i+2}$ (Hz)	8.0	9.0	8.0	9.0	8.0	8.0	8.5	9.0
NH_{i+1}–NH_{i+2}	Medium	Strong	Strong	Strong	Strong	Strong	Weak	Medium
NH_{i+2}–NH_{i+3}	Medium	Weak	Weak	Weak	Strong	Medium	Strong	Medium

[a] Throughout, the terms 'fast', 'medium', and 'slow' in the H/D exchange experiments were relative values for particular compounds based on plots of the extent of exchange versus time. The terms 'strong', 'medium', and 'weak' in ROESY spectra were relative values for particular compounds based on counting contours in the spectra.

Table 3. Key NMR data for some sulfone peptidomimetics in the series **3**–**6**

Parameter[a]	Sulfones							Ideal β-I turn
	3p	**3v**	**3w**	**3x**	**4g**	**5g**	**6g**	
NH_{i+2} T_c (−ppb/K)	3.7	1.4	1.6	0.4	2.4	2.0	2.5	N/A
NH_{i+2} H/D exchange	Slow	Slow	Slow	Slow	Slow	Slow	Slow	N/A
NH_{i+3} T_c (−ppb/K)	1.70	1.4	0.8	0.4	1.5	2.5	2.0	<3.0
NH_{i+3} H/D exchange	Slow	Slow	Slow	Slow	Slow	Slow	Slow	Slow
^3JC$_\alpha$-NH$_{i+1}$ (Hz)	5.5	4.2	5.5	4.8	4.5	5.0	4.5	4.0
^3JC$_\alpha$-NH$_{i+2}$ (Hz)	9.0	8.5	9.0	9.0	8.5	9.0	8.5	9.0
NH_{i+1}–NH_{i+2}	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
NH_{i+2}–NH_{i+3}	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium

[a] Throughout, the terms fast, medium, and slow in the H/D exchange experiments were relative values for particular compounds based on plots of the extent of exchange versus time. The terms strong, medium, and weak in ROESY spectra were relative values for particular compounds based on counting contours in the spectra.

Table 4. Key NMR data for some sulfoxide peptidomimetics in the series **3**–**6**

Parameter[a]	Sulfoxide-*R*			Sulfoxide-*S*			Ideal β-I turn
	3j	**3l**	**3n**	**3k**	**3m**	**3o**	
NH_{i+2} T_c (−ppb/K)	3.7	5.4	4.0	3.7	4.6	4.0	N/A
NH_{i+2} H/D exchange	Fast	Fast	Fast	Slow	Slow	Medium	N/A
NH_{i+3} T_c (−ppb/K)	1.26	1.4	4.0	1.7	1.6	2.0	<3.0
NH_{i+3} H/D exchange	Slow	Medium	N/A	Slow	Slow	Slow	Slow
^3JC$_\alpha$-NH$_{i+1}$ (Hz)	5.0	7.5	7.0	5.5	5.5	5.0	4.0
^3JC$_\alpha$-NH$_{i+2}$ (Hz)	7.5	8.5	8.0	9.0	8.0	8.0	9.0
NH_{i+1}–NH_{i+2}	Weak	Strong	Strong	Medium	Medium	Medium	Medium
NH_{i+2}–NH_{i+3}	Strong	None	None	Medium	Medium	Medium	Medium

[a] Throughout, the terms fast, medium, and slow in the H/D exchange experiments were relative values for particular compounds based on plots of the extent of exchange versus time. The terms strong, medium, and weak in ROESY spectra were relative values for particular compounds based on counting contours in the spectra.

3. Conformational analyses and assignments of absolute configurations of the sulfoxides

The assertion that type I β-turns are favored conformations for the sulfones and one epimer of the sulfoxides is supported by the NMR data compiled in Tables 2–4, CD studies (as previously communicated) and extensive molecular simulations, the end-results of which are summarized in Fig. 3. Comparison of the NMR data collected for the sulfides **3a**, **3g**, **3h**, **3i**, **4a**, **5a**, and **6a** in Table 2 show a wide variance for the parameters. This implies the sulfides have different conformational preferences. There is no correspondence between the data for any one of these compounds and the values expected for an ideal type I β-turn implying these compounds do not have a strong bias to those conformations. Conversely, in Table 3, all the seven sulfones studied give NMR data that corresponds with ideal type I β-turn conformations. A shared conformational bias towards this type of structure is implied. In Table 4, the data for one series of sulfoxide epimers **3j**, **3l**, and **3n** do not compare well with the NMR parameters for and ideal type I β-turn, but the same parameters for the complementary ones **3k**, **3m**, and **3o** do. Throughout, the first series is epimeric with the corresponding sulfoxide in the second series.

Assignment of the *S*-atom configurations of the sulfoxides is difficult because the only physical technique that would unambiguously assign this is single crystal X-ray analyses. Unfortunately, the compounds did not form suitable

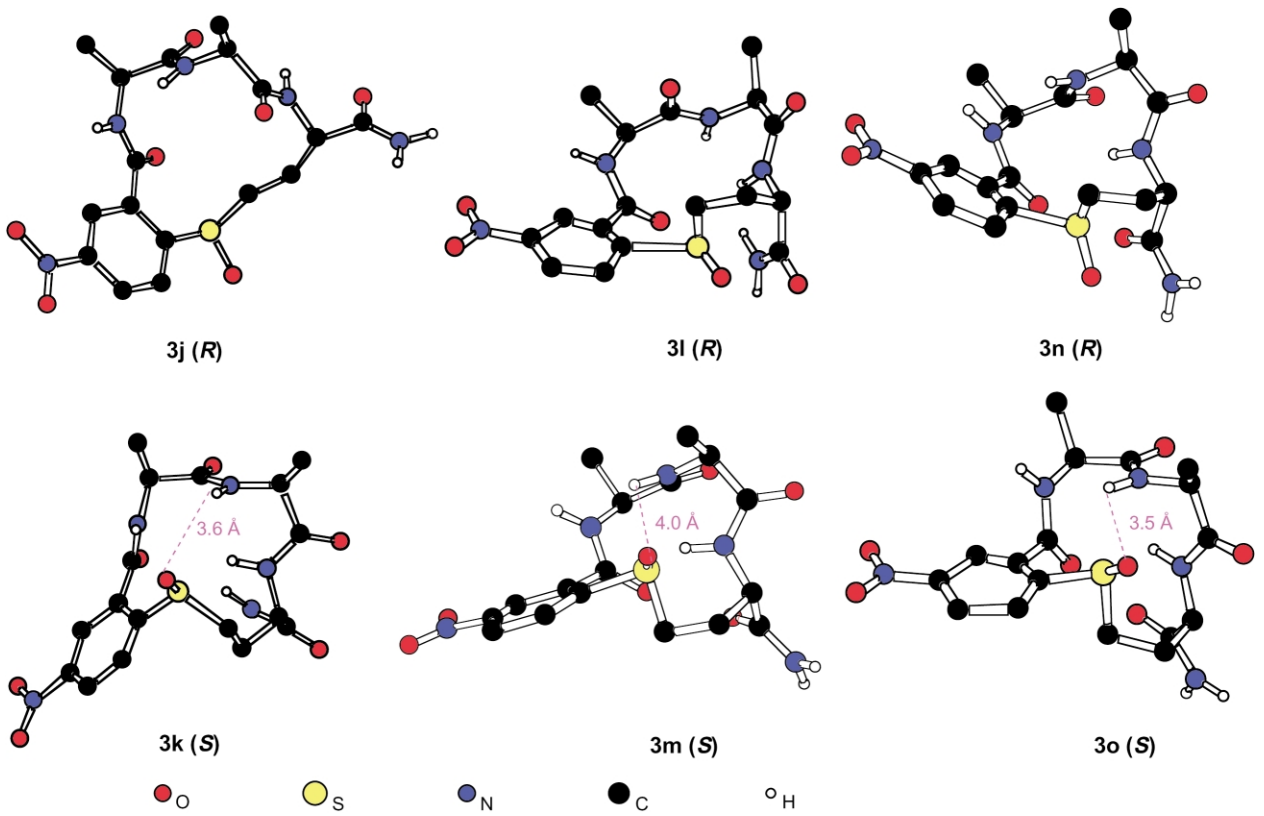


Figure 3. Low energy conformations of three (*R*)-sulfoxides, which do not show β-turn conformations, contrasted with three (*S*)-sulfoxides which do.

crystals. For this reason, definitive proof of the absolute configurations indicated for the sulfoxides in Table 1 was not obtained. To approach this problem, we therefore investigated the unusual idea that configurational assignments of the sulfoxides could be made via conformational analyses. Thus data from the experiments to measure temperature coefficients, H/D exchange rates, coupling constants, ROE close contacts, and CD spectra were interfaced with QMD simulations. These molecular simulations were performed without introduction of any constraints from NMR or other physical measurements. They predict that the (*R*)-sulfoxides in this series do not have preferences for type I β-turn conformations but the (*S*)-sulfoxides do (see Fig. 3 for simulated low energy conformations of six representative sulfoxides). When the NMR data is compared with the simulated low energy conformations, two key observations are made:

- simulations of the (*R*)-sulfoxides indicated these compounds did not have a bias towards β-turn structures, and the favored (non-β-turn) conformations fit well with the physical data for the epimer that did not populate β-turn conformations;
- simulations of the (*S*)-sulfoxides indicated these compounds did have a bias towards β-turn structures, and the favored virtual β-turn conformations fit well with the physical data for the epimer that did populate β-turn conformations.

4. Conclusions

The data presented in Table 1 indicate that peptidomimetics **3**–**6** can be prepared via multi-step solid phase syntheses to give products with high analytical purities. The NMR studies show that the sulfides in this series have various conformational preferences that are not easily recognized, the sulfones tend to adopt type I β-turns, and one of the sulfoxide epimers in each series also has a preference for this type of conformation. Molecular simulations indicate it is the (*S*)-sulfoxides that has a detectable preference for type I turns, but the (*R*)-sulfoxides do not and our assignment of configuration at sulfur is based on this. Configurational assignments based on this type of correlation are not definitive. However, for them to be incorrect, the molecular simulations of both epimers of the sulfoxides would have to be consistently wrong, and the true conformation of the (*R*)-sulfoxide epimer would have to be a type I β-turn that does not emerge from the simulations. We conceive this is possible, but think it is highly unlikely. This is especially so since the molecular simulations predict type I β-turn conformations for the sulfones but not for the sulfides, and both these observations are consistent with the NMR and CD studies. Overall, this work illustrates a rare case where conformational analyses can be used to deduce stereochemical assignments.

5. Experimental

5.1. General methods

All the α-amino acids used were of the L-configuration, except where otherwise indicated. All chemicals were obtained from commercial suppliers and used without

further purification. N^2-(5-Fluoro-2,4-dinitrophenyl)-L-alanine amide (Marfey's reagent), 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *N*-hydroxybenzotriazole (HOBt), di-*iso*-propylethylamine (DIEA), trifluoroacetic acid (TFA), CH_2Cl_2, DMF, thionyl chloride, piperidine and tri-*iso*-propylsilane (TIS) were purchased from Aldrich. Fluoronitrobenzoic acid derivatives chlorides were obtained by refluxing the corresponding fluoronitrobenzoic acid derivative in thionyl chloride for 4 h. TentaGel S RAM Fmoc resin was a gift from Rapp polymere. All protected amino acids used (except *N*-α-Fmoc-*S*-tritylhomocysteine) were purchased from Advanced ChemTech or Novabiochem. Fluoronitrobenzoic acid derivatives (in Fig. 2) were prepared according to literature precedent.[22,23] Reverse phase high performance liquid chromatography (HPLC) was carried out on Vydac C-18 columns of the following dimensions: 25×0.46 cm^2 for analysis, and 25×2.2 cm^2 for preparative work. All HPLC experiments were performed using gradient conditions. Eluents used were solvent A (H_2O with 0.1% TFA) and solvent B (CH_3CN with 0.1% TFA). Flow rates used were 1.0 ml min^{-1} for analytical, and 10 ml min^{-1} for preparative HPLC.

5.2. NMR studies

NMR spectra of the peptidomimetics were recorded on a Varian UnityPlus 500 spectrometer. The concentrations of the samples were approximately 5 mM in DMSO-d_6 throughout. One-dimensional (1D) ^1H NMR spectra were recorded with a spectral width of 8000 Hz, 16 transients, and a 2.5 s acquisition time. Vicinal coupling constants were measured from 1D spectra at 25°C. Assignments of ^1H NMR resonance in DMSO were performed using sequential connectivities. Temperature coefficients of the amide protons were measured via several 1D experiments in the temperature range 25–50°C adjusted in 5°C increments with an equilibration time of more than 20 min after successive temperature steps.

Two-dimensional (2D) NMR spectra were recorded at 25°C with a spectral width of 8000 Hz. Through-bond connectivities were elucidated by COSY, and through-space interactions were identified by ROESY spectra, recorded in 512 t_1 increments and 32 scans per t_1 increment, with 2K data points at t_2. ROESY experiments were performed using mixing times of 200, 300, 400 ms; normally a mixing time of 300 ms was superior. The intensities of the ROESY cross-peaks were assigned as S (strong), M (medium), and W (weak) from the magnitude of their volume integrals.

5.3. CD studies

CD measurements were obtained on an Aviv (model 62 DS) spectrometer. For these experiments the cyclic peptidomimetics were dissolved in H_2O/CH_3OH (80:20 v/v) (c=0.1 mg ml^{-1}, 0.1 cm path length). The CD spectra were recorded at 25°C.

5.4. Quenched molecular dynamics (QMD) studies

CHARMm (version 23.2, Molecular Simulations Inc.) was used for the molecular simulations performed in this work (compounds **1**–**4**). Explicit atom representations were used throughout the study. The residue topology files (RTF) for all the peptidomimetics were built using QUANTA97 (Molecular Simulations Inc.).

QMD simulations were performed using the CHARMm standard parameters. All four molecules were modeled as neutral compounds in a dielectric continuum of 45 (simulating DMSO). Thus, the starting conformers were minimized using 1000 steps of steepest descent (SD) and 3000 steps of the adopted basis Newton–Raphson method (ABNR), respectively. The minimized structures were then subjected to heating, equilibration, and dynamics simulation. Throughout, the equations of motions were integrated using the Verlet algorithm with a time step 1 fs, and SHAKE was used to constrain all bond lengths containing polar hydrogens. Each peptidomimetic was heated to 1000 K over 10 ps and equilibrated for another 10 ps at 1000 K, then molecular dynamics runs were performed for a total time of 600 ps with trajectories saved every 1 ps. The resulting 600 structures were thoroughly minimized using 1000 steps of SD followed by 3000 steps of ABNR until an RMS energy derivative of $\leq$0.01 kcal mol^{-1} $Å^{-1}$ was obtained. Structures with energies less than 3.0 kcal mol^{-1} relative to the global minimum were selected for further analysis.

The QUANTA97 package was again used to display, overlay, and classify the selected structures into conformational groups. The best clustering was obtained using a grouping method based on calculation of RMS deviation of a subset of atoms, in this study these were the ring backbone atoms. Thus, threshold cutoff values between 0.56 and 0.75 Å were selected to obtain families with reasonable homogeneity. The lowest energy from each family was considered as a typical representative of the family as a whole. Additionally, a second approach was also used to obtain a representation of each family. In this alternative protocol, the coordinates of all the heavy atoms in each family were averaged in Cartesian space. The protons were re-built on those heavy atoms using standard geometries for each atom type, then the resulting structures were minimized using 50–100 steps of SD to smooth the bond lengths and angles. Finally, inter-proton distances and dihedral angles from both the lowest energy and the averaged structure were calculated for comparisons with the ROE data.

5.4.1. Bis-*N*-α-Fmoc-homocysteine. A round bottom flask with a magnetic stirrer was charged with 10.6 g (0.100 mol) of sodium carbonate in 60 ml of water, and 2.00 g (4.34 mmol) of bis-*N*-trifluoroacetyl homocysteine (**1**) was added. The solution was stirred at 25°C for 12 h, dioxane (60 ml) was added to the solution, then 3.22 g (9.55 mmol) of *N*-[(9-fluorenylmethoxycarbonyl)-oxy]succinimide (FmocOSu) in 20 ml of dioxane was added to the mixture over 15 min. The mixture was stirred for 4 h at 25°C followed by adding citric acid until a pH of 4 was reached. After removing most of the dioxane in vacuo, 250 ml of ethyl acetate was added, and the layers were separated in a separating funnel. The aqueous phase was extracted with ethyl acetate (3×150 ml). The combined organic layer was washed with water (3×200 ml), saturated NaCl (2×200 ml).

The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to give a shallow yellowish solid. Thoroughly drying the solid yielded 2.78 g of bis-*N*-α-Fmoc-homocysteine as the product in 90% yield. ^{1}H NMR (300 MHz, DMSO-d_6, 25°C) δ 7.90 (d, J=7.2 Hz, 4H), 7.73 (d, J=7.2 Hz, 4H), 7.70 (d, J=7.2 Hz, 2H), 7.42 (t, J=7.2 Hz, 4H), 4.35–4.28 (m, 4H), 4.27–4.20 (m, 2H), 4.15–4.06 (m, 2H), 2.83–2.69 (m, 4H), 2.21–2.07 (m, 2H), 2.04–1.89 (m, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz, 25°C) δ 174.2, 156.9, 145.9, 144.5, 141.4, 128.3, 127.8, 127.5, 125.9, 120.8, 120.6, 66.3, 64.5, 53.2, 50.8, 47.4, 34.8, 30.9. MALDI MS: calcd for [M], 712.83, found 714.02.

5.4.2. *N*-α-Fmoc-homocysteine. A sample of 2.0 g (2.8 mmol) of bis-*N*-α-Fmoc-homocysteine was dissolved in 20 ml of DMF, and 4 ml of water was added. 2.16 g (14.0 mmol) of dithiothreitol (DTT) was added to the solution, stirred at 50°C for 4 h. When TLC showed no starting material remained, 250 ml of water was added to the solution and extracted with ethyl acetate (4×150 ml). The combined organic layer was washed with water (3×150 ml), saturated NaCl (2×150 ml). The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to give a yellowish oil. After flash chromatography with solvents ethyl acetate/hexane (1:2), 1.69 g of *N*-α-Fmoc-homocysteine was obtained in a yield of 85%. ^{1}H NMR (300 MHz, DMSO-d_6, 25°C) δ 7.91 (d, J=7.2 Hz, 2H), 7.74 (d, J=7.2 Hz, 2H), 7.68 (d, J=7.2 Hz, 1H), 7.43 (t, J=7.2 Hz, 2H), 7.35 (t, J=7.2 Hz, 2H), 4.32 (m, 2H), 4.24 (m, 1H), 4.15 (dd, J=7.2, 16 Hz, 1H), 2.53 (m, 2H), 2.35 (t, J=7.2 Hz, 1H), 1.94 (m, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz, 25°C) δ 174.6, 157.3, 144.7, 141.5, 128.4, 127.8, 126.0, 120.9, 66.5, 53.5, 47.5, 35.7, 21.6. ESI-MS: calcd for [M], 357.42, found 358.66 [M+H$^+$], 380.48 [M+Na$^+$].

5.4.3. *N*-α-Fmoc-*S*-tritylhomocysteine (2). *N*-α-Fmoc-homocysteine (0.89 g, 2.50 mmol), triphenylmethanol (0.65 g, 2.50 mmol) were dissolved in 200 ml of anhydrous CH_2Cl_2, followed by adding 0.5 equiv. of trifluoroacetic acid (96 μl, 1.25 mmol), stirred at 25°C for 6 h. Pyridine was added to neutralize the trifluoroacetic acid, the organic phase was washed with water to remove the pyridine salt, evaporating the CH_2Cl_2, and the crude product was purified by flash chromatography with solvents ethyl acetate/hexane (1:2.5). 0.9 g of **2** was obtained in a yield of 60%. $[\alpha]_D^{20}$=+12° (c=1.0, CH_3OH). ^{1}H NMR (300 MHz, DMSO-d_6, 25°C) δ 7.91 (d, J=7.2 Hz, 2H), 7.72 (d, J=7.2 Hz, 2H), 7.56 (d, J=7.2 Hz, 1H), 7.44 (t, J=7.2 Hz, 2H), 7.36–7.20 (m, 17H), 4.31 (m, 2H), 4.23 (m, 1H), 4.00 (m, 1H), 2.19 (m, 2H), 1.70 (m, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz, 25°C) δ 174.8, 156.9, 145.1, 141.5, 129.8, 128.7, 128.4, 127.8, 127.4, 126.0, 120.9, 66.8, 66.4, 53.6, 47.4, 30.3, 28.8. ESI-MS: calcd for [M], 599.74, found [M+H$^+$], 600.58, [M+Na$^+$], 622.5.

5.5. Determination of the enantiomeric purity of *N*-α-Fmoc-*S*-tritylhomocysteine (2)

A sample of 20 mg of **2** was dissolved in 1 ml of ethyl acetate, and then 33 μl (20 equiv.) of piperidine was added, followed by precipitating with 2 ml of hexane. 10 mg of *S*-tritylhomocysteine was obtained with high purity (analytical HPLC checked). Following Marfey's test procedures,[24] N^2-(5-fluoro-2,4-dinitrophenyl)-L-alanine amide was coupled to the *S*-tritylhomocysteine to form a L,L-diastereomer, analytical HPLC showed a homogeneous single peak. Retention time 21.2 min indicating the high enantiomeric purity of the *N*-α-Fmoc-*S*-tritylhomocysteine (**2**). As a control, a sample of *S*-tritylhomocysteine which was prepared according to literature,[13] but was known to be a mixture of enantiomers, was also coupled to the N^2-(5-fluoro-2,4-dinitrophenyl)-L-alanine amide, analytical HPLC showed two peaks, retention times are 21.2 and 22.1 min, respectively, with a ratio of 2:1, which were the L,L- and L,D-diastereomers, indicating the racemization.

5.6. General experimental for syntheses of the peptidomimetics sulfides: synthesis of compound 3a

TentaGel S RAM Fmoc resin (100 mg, 0.220 mmol g^{-1}) was swelled in DMF (10 ml g^{-1}) in a polypropylene syringe for 30 min, then rinsed with DMF (2×10 ml g^{-1}, for each washing cycle throughout). The Fmoc protecting group on the Rink handle was removed by treating the resin with 20% piperidine in DMF (2×15 min). After the resin was rinsed with DMF (3×), CH_3OH (3×), and CH_2Cl_2 (3×), Fmoc-homoCys(Trt)-OH (**2**) (4 equiv.), HBTU (4 equiv.), HOBt (4 equiv.), and DIEA (6 equiv.) were added in 5 ml of DMF. After 2 h of gentle shaking, a ninhydrin test on a small sample of beads gave a negative result. The reaction mixture was drained and the resin was rinsed with DMF (4×). The above deprotection/coupling cycles were repeated to introduce Fmoc-Lys(Boc)-OH and Fmoc-Glu(O^{t}Bu)-OH consecutively. The 2-fluoro-5-nitrobenzoic acid moiety was introduced to the N-terminus of the tripeptide-resin by treating with 2-fluoro-5-nitrobenzoyl chloride (2 equiv.) and ^{i}Pr$_2$NEt (4 equiv.) in 1 ml of CH_2Cl_2 for 1 h. The side-chain protecting group (trityl) of homoCys was removed by treatment with 3% TFA and 4% TIS in CH_2Cl_2 (5×5 min). After, the resin was rinsed with CH_2Cl_2 (3×), CH_3OH (3×), and DMF (3×), the macrocyclization step was carried out by treating the supported peptide with 5 equiv. K_2CO_3 in DMF at 25°C with gentle shaking for 36 h. The peptide-resin was washed with DMF (2×), H_2O (3×), DMF (3×), H_2O (2×), CH_3OH (3×), CH_2Cl_2 (3×), and then dried in vacuo for 4 h. The peptide was cleaved from the resin by treatment with a 5 ml mixture of 95% TFA, 2.5% TIS and 2.5% H_2O for 2 h. The cleavage solution was separated from the resin by filtration. After most of the cleavage cocktail was evaporated by passing N_2, the crude peptide was precipitated using anhydrous ethyl ether, then dissolved in H_2O, and lyophilized to give the crude product. Preparative HPLC (Beckman System, 5–90% B in 40 min) was carried out to provide a slight yellowish powder (3.8 mg, 32%) of **3a**. ^{1}H NMR (500 MHz, DMSO-d_6, 25°C), see Table of ^{1}H NMR (500 MHz) chemical shifts (ppm) for **3a**. Analytical HPLC: single main peak, retention time 14.2 min (5–70% B in 30 min). MALDI MS: calcd for $C_{22}H_{30}N_6O_8S$, 538.5, found 539.6 [M+H$^+$]. 560.5 [M+Na$^+$] and 577.4 [M+K$^+$].

5.7. General experimental for syntheses of the peptidomimetics sulfones: synthesis of compound 3p

10 mg of **3a** was dissolved in 2 ml formic acid (90% in H_2O), 0.4 ml H_2O_2 (30% in H_2O) was added, stirred overnight at 25°C. Evaporated most of solvent under

vacuum and dissolved in H_2O, lyophilized to give the crude product. Preparative HPLC (Beckman System, 5–90% B in 40 min) was carried out to provide a white powder (6.6 mg, 65%, based on **3a**) of **3p**. ^{1}H NMR (500 MHz, DMSO-d_6, 25°C), see Table of ^{1}H NMR (500 MHz) chemical shifts (ppm) for **3p**. Analytical HPLC: single main peak, retention time 13.1 min (5–70% B in 30 min). MALDI MS: calcd for $C_{22}H_{30}N_6O_{10}S$, 570.5, found 571.6 ([M+H$^+$]), 593.7 ([M+Na$^+$]).

5.8. General experimental for syntheses of the peptidomimetics sulfoxides: synthesis of compounds 3j and 3k

Compound **3a** (30 mg) was dissolved in 8 ml CH_3CN/H_2O (3:5 v/v), 240 μl (2 equiv.) of $NaIO_4$ solution (10 mg ml^{-1} in H_2O) was added, stirred at 25°C for 24 h, HPLC analysis showed only two main peaks, retention time 11.0 and 12.1 min with ratio of 1:2. HPLC purification gave 7.0 mg (23%, based on **3a**) and 14.0 mg (45%, based on **3a**) of **3j** and **3k**. ^{1}H NMR (500 MHz, DMSO-d_6, 25°C), see Table of ^{1}H NMR (500 MHz) chemical shifts (ppm) for **3j** and **3k**. MALDI MS: calcd for $C_{22}H_{30}N_6O_9S$, 554.5, found 555.4 ([M+H$^+$]) for both **3j** and **3k**.

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