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Structural elucidation of the β-turn inducing (*S*)-[3-amino-4-oxo-2,3-dihydro-5*H*-benzo[*b*][1,4]thiazepin-5-yl] acetic acid (DBT) motif

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Abstract—X-ray diffraction analysis of Boc-DBT-NH_2 (DBT = (*S*-[amino]-5-carbethoxymethyl-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one) showed that this constrained dipeptide mimetic adopts a type II′ β-turn in the solid state. IR and NMR studies indicated that the folded conformation is retained in solution.

Incorporation of conformational constraints into biologically active peptides to limit the number of available conformations and increasing metabolic stability, potency, selectivity and bioavailability has been the focus of extensive studies over the last years.[1] Conformationally restrained surrogates have been successfully used in the design and synthesis of enzyme inhibitors and antagonists of peptide hormone receptors.[2] Alternatively, constrained non-peptidic templates that mimic or stabilise architectural elements of peptides such as β-turn motifs have received a great deal of attention.[1] Our approach for the design of peptide hormone bradykinin (BK: *H*-Arg[1]-Pro[2]-Pro[3]-Gly[4]-Phe[5]-Ser[6]-Pro[7]-Phe[8]-Arg[9]-OH) analogues, was to replace the dipeptide Pro[7]-Phe[8]- in BK or -D-Tic[7]-Oic[8]- in its potent antagonist HOE 140[3] (*H*-D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Thi[5]-Ser[6]-D-Tic[7]-Oic[8]-Arg[9]-OH) by conformationally restrained surrogates.[4] We showed that substitution of Pro-Phe in BK or D-Tic-Oic in HOE140 by the non-peptidic scaffold 3-*S*-[amino]-5-carbethoxymethyl-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one (DBT) resulted in full potent and selective bradykinin B_2 receptor agonists with a high affinity for the human receptor. It has been suggested that the high affinity for B_2 receptors of bradykinin analogues (including HOE140) is related to their high propensity to adopt a C-terminal β-turn conformation spanning the C-terminal tetrapeptide. We concluded from the biological results that the DBT moiety should constitute a good β-turn mimetic. This motif was used as a constrained dipeptide mimetic, but no structural determination has been performed to date. We therefore investigated the conformational analysis of Boc-DBT-NH_2 (Scheme 1) in the solid state by X-ray diffraction, and in organic solution (CH_2Cl_2, $CHCl_3$ and DMSO) by IR and proton NMR spectroscopies.

After reinvestigation of the procedure proposed by Slade et al.,[5] Boc-DBT-OH was obtained by a five-step procedure starting from the condensation of Boc-D-Cys-OH and 1-fluoro-2-nitrobenzene.[6] Condensation of Boc-DBT-OH with NH_4OH was carried out through activation with isobutyl chloroformate in the presence of *N*-methylmorpholine to afford Boc-DBT-NH_2 in quantitative yield (Scheme 1).

The seven-membered benzothiazepinone ring, which is composed of the two planar amide and aromatic fragments, may adopt two symmetrical conformations allowing an axial (A) or equatorial (E) orientation of the Boc-amino group (Fig. 1).[7] They can be discriminated by the rotational state of the Cys C^{α}–C^{β} bond, and therefore the H^{α}/H^{β} vicinal coupling constants,

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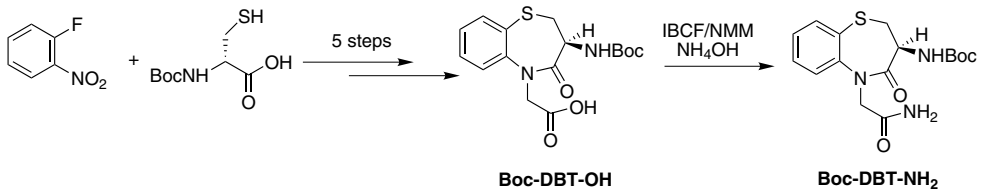

Scheme 1. Synthesis of Boc-DBT-NH_2.

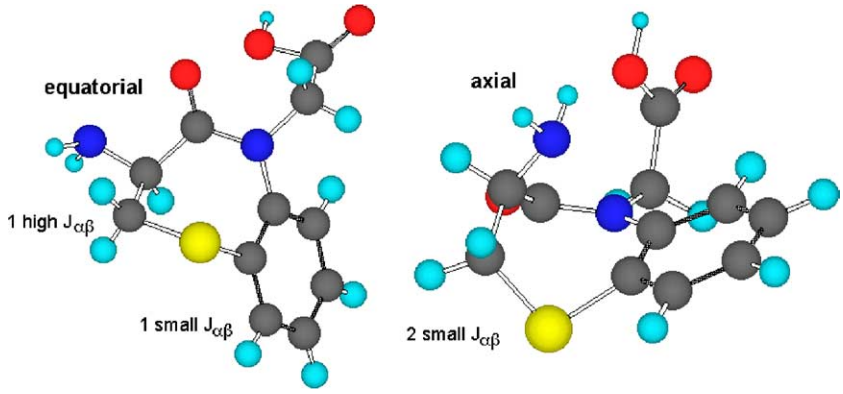

Figure 1. The two possible axial and equatorial conformations of the DBT motif.

corresponding to the Cys-χ^1 value of about −60° (A) or 180° (E).

Solid state: single crystals of Boc-DBT-NH_2, suitable for X-ray diffraction,[8] were obtained from diethyl ether solution. Structure analysis showed that the studied dipeptide mimetic adopts a folded conformation that is stabilised by an intramolecular $i + 3 \rightarrow i$ H-bond (N···O = 3.12 Å) closing a 10-membered cycle, and the benzothiazepinone ring assumes the E form ($\chi^1 = -168°$) (Fig. 2a). The main torsional angles[9] ('ϕ_i' = 66°, 'ψ_i' = −144°, 'ϕ_{i+1}' = −83°, 'ψ_{i+1}' = 7°) reveal a type II′ β-turn structure which is quite superimposable to II′ β-folded Ac-D-Tic-L-Oic-NH-Me[10] in HOE 140[11]

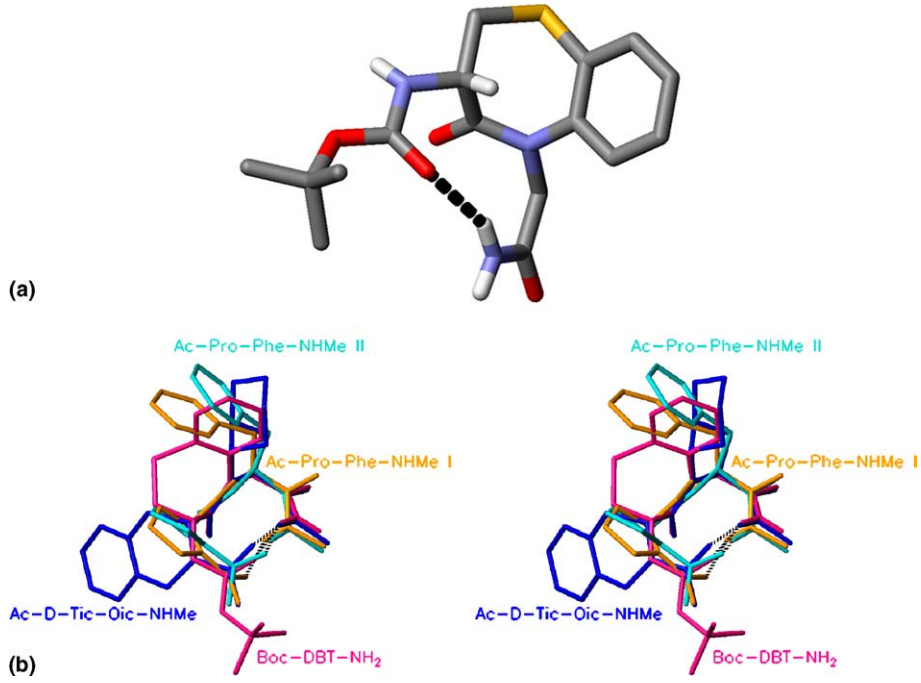

Figure 2. Crystal molecular structure of Boc-DBT-NH_2 in the β II′-folded conformation (a) and stereoview of the superimpositions of Boc-DBT-NH_2 (pink) with the β II′-folded Ac-D-Tic-L-Oic-NHMe dipeptide (blue), the β I-folded (orange) and β II-folded (cyan) Ac-L-Pro-L-Phe-NHMe model dipeptide (b).

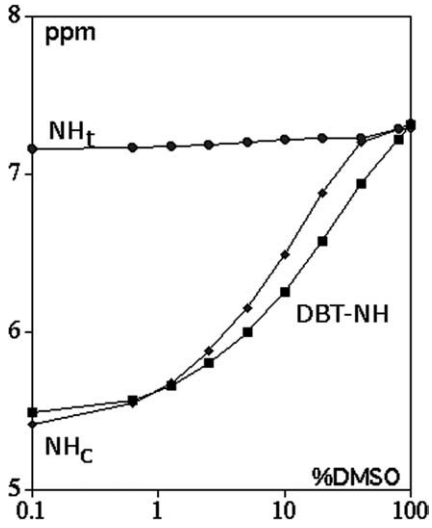

Figure 3. Variation of the NH NMR-resonances in $CDCl_3$/DMSO-d_6 mixtures.

with a root-mean-square deviation (rmsd) of 0.15 Å (Fig. 2b). In this superimposition, the DBT aromatic ring occupies the 'free' space between the Tic and Oic side chains. Boc-DBT-NH_2 has also been compared with the model dipeptide Ac-L-Pro-L-Phe-NHMe[12] folded in both types I and II β-turns (rmsd of 0.57 Å and of 0.55 Å, respectively). Optimal superimpositions (Fig. 2b) have been obtained with the g^- rotamer[13] for the side chain of the Phe residue.

Solution state: in CH_2Cl_2, the IR spectrum of Boc-DBT-NH_2 presents a single, free absorption at 3425 cm^{-1} for the Boc-NH, and two sets of NH_2 stretching absorption with very different intensities. The minor one at 3514 cm^{-1} (asymmetrical vibration) and 3406 cm^{-1} (symmetrical vibration) is typical of a free NH_2 group whereas the major one at 3480 and 3349 cm^{-1} denotes an intramolecular NH_2 to Boc-CO H-bond. Taking the NH proton resonances in $CDCl_3$ as a reference, the chemical shifts due to DMSO solvation reveal the solvent accessibility for both Boc-NH (1.83 ppm) and NH_c (1.92 ppm), and the solvent protection for NH_t (0.13 ppm) (Fig. 3).[14] Moreover, the medium and large H^{α}/H^{β} vicinal coupling constants (6.8 and 11.2 Hz) are in favour of conformation E for the benzothiazepinone ring, with an *anti* orientation for the Cys nitrogen and sulfur, and are compatible with a type II′ β-turn (Fig. 2a). All these results indicate that the folded conformation of Boc-DBT-NH_2 is retained in solution, even in DMSO where the typical H-bonded NH_t absorption is still observed at a higher frequency than that for the two DMSO-solvated NHs (3270 cm^{-1}).

Previous biological results obtained by incorporation of DBT motif in bradykinin analogues suggested that it should constitute a good mimetic of a β-turn. X-ray, IR and NMR study confirm the type II′ β-turn structure of this motif which appears to be an efficient β-turn surrogate in peptides and proteins.

References and notes

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7. Molecular modelling using MM2 calculations in CS Chem3D Pro package.
8. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 243845. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk]. $T = 100(2)$ K; orthorhombic, $P2_12_12_1$; $a = 10.3308(5)$ Å, $b = 11.3122(5)$ Å, $c = 14.5723(7)$ Å; $Z = 4$; $D_{calcd} = 1.371$ g cm^{-3}; wavelength 0.71073 Å.
9. The torsional angles of DBT are defined with reference to homologous peptide.
10. Ac-D-Tic-L-Oic-NH-Me has been modelled from the solution structure of HOE140 using *WeblabviewerPro 3.5*, MSI, 1999.
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12. Ac-L-Pro-L-Phe-NHMe has been built using the torsional angles for an ideal β I-turn: $(\varphi,\psi)_{i+1} = (-60,-30)$; $(\varphi,\psi)_{i+2} = (-90,0)$; and using the torsional angles for an ideal β II-turn: $(\varphi,\psi_{i+1}) = (-60,120)$; $(\varphi,\psi)_{i+2} = (80,0)$. The modellisation has been done using *WeblabviewerPro 3.5*.
13. According to the IUPAC-IUB Commission, g^- rotamer is the rotational state of the C^{α}–C^{β} bond defined by a χ^1 dihedral angle of about −60°.
14. NH_c and NH_t, respectively, denote the proton in the cis and trans position with reference to the carbonyl.