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The Solution Conformation of 1-(3,5-Dimethylphenyl)methyl-3(S)-(1H-indol-3-yl)methyl-6(S)-phenylmethyl-2,5-piperazinedione (1): An NMR and Molecular Modelling Study

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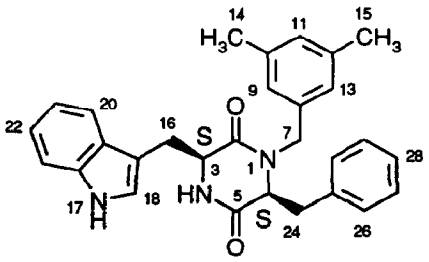
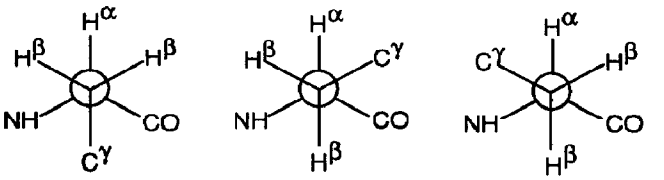
Abstract: Comparison of NMR data with structures from molecular modelling of 1-(3,5-dimethylphenyl)methyl-3(S)-(1H-indol-3-yl)methyl-6-(S)-phenylmethyl-2,5-piperazinedione (1) showed it to have a specific solution conformation with the phenyl ring over the diketopiperazine ring and the tryptophan sidechain in the [G^-_0] rotamer.

Piperazine-2,5-diones (diketopiperazines, DKPs) have been extensively studied[1-5] (see ref. 4 for review) in part because the conformational restrictions of the DKP ring make them attractive peptide models for studying side-chain/side-chain and side-chain/backbone interactions. Of particular interest are the DKPs containing one or more aromatic amino acid residues due to a propensity for a parallel orientation of the aromatic and DKP rings. We wish to report the unusual solution conformation of an N-benzylated cyclo[(L)-Phe-(L)-Trp] dipeptide (1) having a specific orientation of the tryptophan indole moiety as determined from the analysis of high resolution NMR data in conjunction with molecular modelling.



(1)

1-(3,5-Dimethylphenyl)methyl-3(S)-(1H-indol-3-yl)methyl-6-(S)-phenylmethyl-2,5-piperazinedione (1) was prepared by condensation of (L)-phenylalanine methyl ester hydrochloride with 3,5-dimethylbenzaldehyde, reduction of the imine, condensation with N-Boc (L)-tryptophan, hydrolysis of the Boc protecting group and cyclisation under basic conditions. NMR and mass spectrometric data were obtained.* X-ray data was not available as the compound was an amorphous powder but the solution conformation was determined from the NMR data in conjunction with molecular modelling. A set of 800 conformations was generated using distance geometry (DGEOM) and these were optimised and grouped into families (OPTI_CONF and ANAL_CONF in AMF).[17] The lowest energy members of each family were then compared to the NMR data for consistency with the observed δ, ^3J and nOe enhancements.



Rotamers	G^+	T	G^-
Conformations	F	E_o	E_n
χ_1	60^o	180^o	$(-60^o)\ 300^o$

Figure 1: Newman projection of the rotational isomers about the C^{α}-C^{β} bond.

The coupling constants $^3J_{3,16a}$=11.24Hz and $^3J_{3,16b}$=2.56Hz were consistent with both the T rotamer (Eo, χ_1=180°) or the G^- rotamer [E_n, χ_1= -60°(300°)] (Figure 1) of the Trp sidechain.[5-8] The nOe data indicated small internuclear distances (<3.0A) for 18H-16H_a, 18H-4NH, 20H-3H and 20H-16H_b (all observed) and larger internuclear distances (>4.0A) for 18H-3H, 18H-16Hb, 20H-4NH and 20H-16Ha (not observed), consistent only with the G^- rotamer. The magnitudes of $^3J\alpha,\beta$ were consistent with almost exclusive population of the G^- rotamer.[7] Consideration of the corresponding typical internuclear distances from model G^- conformations with the 6-membered ring of the indole oriented either towards O2 (G^-_o) or N4 (G^-_n) [(G^-_o/G^-_n):2.7/4.0, 3.0/4.2, 2.7/4.3, 2.6/4.0, 4.4/2.7, 3.9/2.7, 4.5/3.1 and 4.1/2.4 A] indicated that only G^-_o was consistent with the observed nOe data, thus defining the relative orientation of the tryptophan sidechain and diketopiperazine ring (DKP) with the indole moiety in one specific orientation (G^-_o) (Figure 2). The predominant rotamer around the phenylalanine $\alpha\beta$ bond was determined from the small and equal values of $^3J_{6,24a}$ and $^3J_{6,24b}$ to be G^+ (F, χ_1= -60°)[2,4,5] with the phenyl ring lying face-to-face over the DKP ring. The anomalously highfield shift of 16H_a (δ 1.12ppm) could be accounted for by shielding due to the ring current of the phenyl ring lying close and centrally face-on to this proton, consistent with the G^+ rotamer.[9,10] Finally, nOe data, including {24H_b}7H_a[S], {6H}7H_a[M], {6H}9H/13H[S/M] and {3H}9H/13H[M], proved the N1 aryl substituent to be on the other face of the DKP ring from the Phe residue phenyl. The very short internuclear distance required to give a strong nOe between 7H_a and 24H_b, both of which have a competing geminal methylene proton, was measured as 2.2-2.3A in the modelled conformers consistent with the nOe data above. The degree of buckle of the diketopiperazine ring from ^3JNH,α and $^5J\alpha,\alpha$ (both <<1Hz) was predicted to be β<0, in a 'flagpole boat' conformation with both the phenylalanine and tryptophan side chains axial consistent with the overall conformation derived from NMR data.[4,11-13]

The solution conformations of several aryldiketopiperazines have been studied by NMR. In general, the aromatic moiety is oriented over the DKP ring in monoaromatic cases and this is also true for diaromatic DKPs, although in the case of symmetrical (L,L)-diaryl DKPs there is evidence for π-π interactions between the face to face aromatic rings [cyclo(L-X)$_2$ where X=Phe, Trp or Tyr].[1,14] The stabilisation of the aryl-DKP interaction has been ascribed to short range effects such as dipole-induced dipoles[1,6] or van der Waals dispersion forces.[4]

The conformation derived from analysis of the NMR data of (1) shows the phenylalanine aromatic ring interacting with the DKP ring whilst the tryptophan sidechain is in a very specific conformation (G^-, χ_1= -60°), away from the DKP ring and, unusually, with the indole moiety essentially in only one orientation around the $\beta\gamma$ bond (G^-_o). The aromatic moieties are not stacked face-to-face unlike the situation in symmetrical cyclo[L,L]DKPs of aromatic amino acids.[14] Studies of α-deuterium labelled Phe-X diaromatic DKPs have shown that the order of preference for folding over the DKP ring is Trp > Phe.[15] However, in (1) this is reversed with the Phe residue interacting exclusively with the DKP. In the N-alkylated cyclo[L-Phe, L-

MeNPhe] the phenyl ring of the N-methylated residue interacts with the DKP ring.[16] Similarly, the N-aryl substituent of (1) clearly strongly influences the observed conformation causing the phenyl ring rather than the indole to interact with the DKP, in contrast to the molecule where there is no N-aryl substituent.[15]

To investigate the possible reasons for the predominance of a single orientation of the indole ring a comparison of conformer pairs from DGEOM calculations with the indole ring either G^-_o or G^-_n was made. This showed that the calculated energy terms [Optimol using MM2X][17] consistently differed by only ca. 0.5kcal mol^{-1} in the net VDW term in favour of the conformer observed in solution. This would seem to be an insufficiently large difference to explain the apparent bias in the indole orientation (G^-_o) but this bias is strongly supported by the nOe data which gives enhancements corresponding to the G^-_o rotamer but not the G^-_n rotamer.

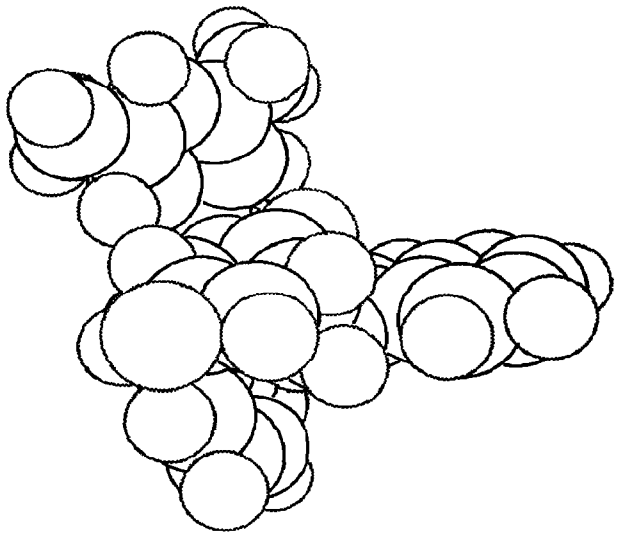


Figure 2: 1-(3,5-Dimethylphenyl)methyl-3(S)-(1H-indol-3-yl)methyl-6-(S)-phenylmethyl-2,5-piperazinedione (1) .[G^-_o] conformer

In summary, analysis of NMR data (δ, ^3J and nOe) of (1) has provided a remarkably well defined solution conformation which is agreement with the lowest energy conformations generated from optimised DGEOM calculations (Fig.1) and shows the strong influence of an N-aryl substituent on the conformation as well as an unusually specific preference in the orientation of the tryptophan indole ring.

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***Spectral data:**

EI+ mass m/z: 451.2275 (M^+). $C_{29}H_{29}N_3O_2$ requires m/z 451.2260.

NMR spectral assignments were made on the basis of COSY-45, DEPT-135, HMQC and HMBC.

^{1}H NMR ($CDCl_3$, 360MHz, 300K, J in Hz) δ_H 1.12 (1H, dd, J14.21, 11.24, $16H_a$), 2.29 (6H, s, $14CH_3$, $15CH_3$), 3.14 (1H, m, $24H_a$), 3.15 (1H, m, $16H_b$), 3.24 (1H, dd, J14.12, 4.47, $24H_b$), 3.86 (1H, d, J14.57, $7H_a$), 4.10 (1H, ddd, J11.20, 2.56, 2.56, 3H), 4.18 (1H, dd, 4.05, 4.05, 6H), 5.65 (1H, bs, 4NH), 5.68 (1H, d, J14.58, $7H_b$), 6.76 (1H, d, J2.17, 18H), 6.87 (2H, s, 9H, 13H), 6.94 (1H, s, 11H), 7.10 (1H, dd, J7.92, 7.92, 21H), 7.19 (2H, d, 26H, 30H), 7.19 (1H, dd, 22H), 7.32 (1H, dd, 28H), 7.33 (1H, d, 23H), 7.43 (2H, dd, J7.11, 7.11, 27H, 29H), 7.52 (1H, d, J7.86, 20H), 8.04 (1H, bs, 17NH).

^{13}C NMR ($CDCl_3$, 90MHz, 300K) δ 21.26 (q, C14, 15), 30.95 (t, C16), 36.26 (t, C24), 46.60 (t, C7), 55.48 (d, C3), 59.03 (d, C6), 110.18 (s, C19), 111.35 (d, C23), 118.80 (d, C20), 119.91 (d, C21), 122.60 (d, C22), 123.41 (d, C18), 126.39 (d, C9, C13), 126.44 (s, C19a), 127.70 (d, C28), 128.98 (d, C27, 29), 129.90 (d, C11), 130.53 (d, C26, 30), 134.96 (s, C8), 135.29 (s, C25), 136.49 (s, C23a), 138.65 (s, C10, 12), 165.71 (s, C2), 166.10 (s, C5).

The nOe data obtained was qualitatively analysed according to the relative intensity of the enhancements as either strong [S], medium [M] or weak [W]:

{$16H_a$}:$16H_b$[S], 18H[M];

{$16H_b$, $24H_a$}:$16H_a$[S], $24H_b$[S], 3H[M], 6H[M], 26H/30H[M], 20H[M];

{$24H_b$}:$24H_a$[S], $7H_a$[S], 6H[S], 26H/30H[M/W];

{3H}:$16H_b$[M], 4NH[S/M], 9H/13H[M/W], 20H[S];

{6H}:$24H_a$[M/W], $24H_b$[M/W], $7H_a$[M], 9H/13H[S/M]

{4NH}:3H[S], 18H[M/W];

{$7H_b$}:$7H_a$[S], 9H/13H[M];

{9H/13H}:$14CH_3$/$15CH_3$[S], $7H_a$[S/M], $7H_b$[M/W], 6H[M/W];

{$14CH_3$/$15CH_3$}:9H/13H[S], 11H[S].

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