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## A Crystal Structure Determination for Tyr-D-Nle-Gly-Phe-NleS [NleS = $MeCH_2CH_2CH_2CH(NH_2)SO_3H$]: an Active Synthetic Enkephalin Analogue

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A crystal structure determination for Tyr-D-Nle-Gly-Phe-NleS [NleS = $MeCH_2CH_2CH_2CH(NH_2)SO_3H$] provides two examples of a 2′-β-bend conformation for a biologically active, synthetic enkephalin analogue.

The synthetic pentapeptide analogue L-tyrosyl-D-norleucyl-glycyl-L-phenylalanyl-L-α-aminopentanesulphonic acid (Tyr-D-Nle-Gly-Phe-NleS) (**1**) has been found to display enkephalin-like activity.[1] An interesting consequence of its chemical structure is that the introduction of a chiral residue at position 2 of the sequence significantly reduces the conformational freedom of this pentapeptide relative to the natural enkephalins (*e.g.* Tyr-Gly-Gly-Phe-Leu, Leu$^5$-enkephalin). We felt that a crystal structure determination for (**1**) would be particularly valuable since Leu$^5$-enkephalin has been found to display very different conformations in two crystalline modifications.[2,3] We report the crystal structure of (**1**) in which there are two molecules of the pentapeptide analogue per asymmetric unit and compare their conformations with eight examples available for Leu$^5$-enkephalin.

Crystals of (**1**) were obtained by slow evaporation of an aqueous ethanol solution: monoclinic, space group $P2_1$, $a$ = 8.588(7), $b$ = 30.265(38), $c$ = 15.024(18) Å, β = 96.94(7)° (at *ca.* 120 K); the composition of the asymmetric unit is: $(C_{31}H_{45}N_5O_7)_2 \cdot 5H_2O \cdot C_2H_5OH$. Diffraction intensities were measured from the cooled crystal to $2\theta_{max.} = 50°$ (monochromatized Mo-$K_\alpha$ radiation) with a Syntex P1 autodiffractometer (equipped with a Syntex LT-1 low temperature device) operating in the ω-scan mode with a scan rate of 0.5—24.0° min$^{-1}$. Of 6944 unique reflections measured, 4307 had $I \geq 3\sigma(I)$. The initial structural model was determined by direct methods[4] and developed[5] by difference Fourier and least squares refinement techniques; 5834 reflections contributed to the refinement of 882 variables to give $R$ = 0.092.†

The two peptide analogue molecules per asymmetric unit display similar 2′-β-bend conformations (Figure 1). Both molecules display an intramolecular hydrogen bond between the tyrosine carbonyl oxygen atom and the amide hydrogen atom of phenylalanine. In one molecule (**1a**) there is a second intramolecular hydrogen bond between the protonated amino group of tyrosine and the carbonyl oxygen atom of phenylalanine that is not present in the second molecule; the

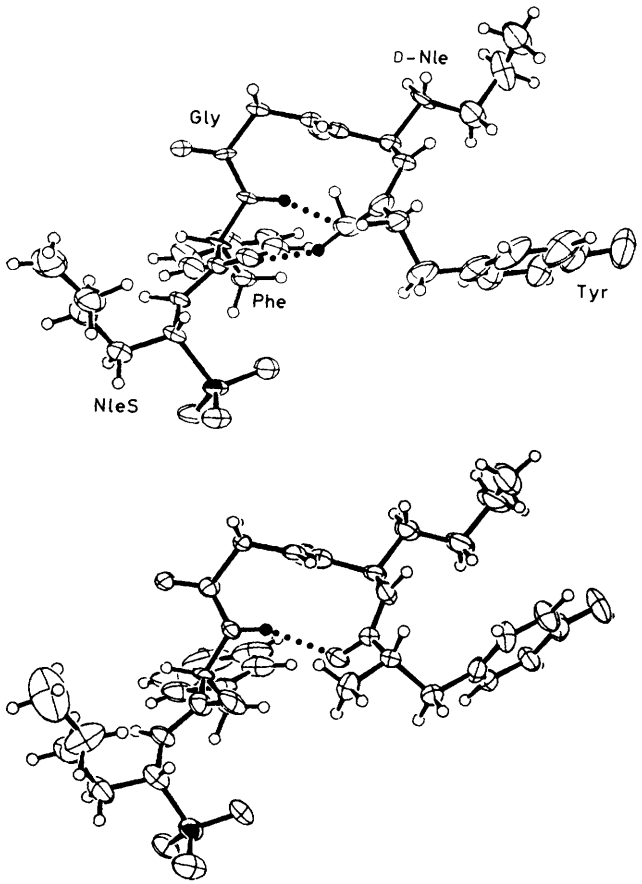


**Figure 1.** ORTEP-II[7] plots illustrating the conformations for the two independent molecules of Tyr-D-Nle-Gly-Phe-NleS in the crystallographic unit. Hydrogen bonds are illustrated with dotted lines. The amino acid sequence is labelled for molecule (**1a**).

† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

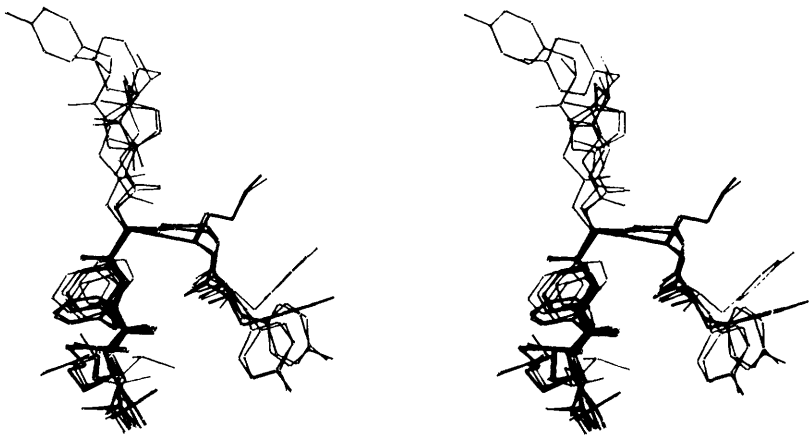

**Figure 2.** A stereoscopic superposition diagram[8] for the two molecules of Tyr-D-Nle-Gly-Phe-NleS, four molecules of Leu$^5$-enkephalin reported by Blundell *et al.*,[2] and the four molecules of Leu$^5$-enkephalin reported by Karle *et al.*[3]

respective N · · · O distances are 2.86(1) Å for (**1a**) and 3.23(1) Å for (**1b**). The conformations in this crystal structure are consistent with that proposed by Casey *et al.*,[6] based on a 400 MHz $^1$H n.m.r. study of (**1**) dissolved in $Me_2SO$.

Space limitations preclude a detailed comparison of the conformations of (**1**) with those reported for Leu$^5$-enkephalin. However, a good qualitative impression of the similarities and differences can be achieved from examination of the stereoscopic superposition diagram[8] presented in Figure 2. This figure also illustrates the marked differences in conformation adopted by Leu$^5$-enkephalin in crystals obtained from aqueous alcohol[2] (β-bend) and from aqueous dimethylformamide (DMF)[3] (extended backbone). A second crystal modification of (**1**) has been obtained from aqueous DMF and is presently under investigation.

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## References

1 S. Bajusz, A. Z. Rónai, J. I. Székely, A. Turán, A. Juhász, A. Patthy, E. Miglécz, and I. Berzétei, *FEBS Lett.*, 1980, **117**, 308.
2 T. L. Blundell, L. Hearn, I. J. Tickle, R. A. Palmer, B. A. Morgan, G. D. Smith, and J. F. Griffin, *Science,* 1978, **205**, 1214.
3 I. Karle, J. Karle, D. Mastropaolo, A. Camerman, and N. Camerman, *Acta Crystallogr., Sect. B,* 1983, **39**, 625.
4 G. M. Sheldrick, Program 'SHELXS-84,' Universität Göttingen, 1984.
5 J. M. Stewart, P. A. Machin, C. W. Dickinson, H. L.-Ammon, H. Flack, and H. Heck, 'XRAY Version of 1976,' Technical Report TR-446, University of Maryland Computer Center, College Park Maryland, U.S.A., 1976.
6 S. Bajusz and A. F. Casey, *Org. Magn. Reson.*, 1982, **22**, 395.
7 C. K. Johnson, 'ORTEP-II, A FORTRAN Thermal Ellipsoid Plot Program for Crystal Structure Illustrations,' Report No. ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A., 1971.
8 M. N. Liebman, in 'Molecular Structure and Biological Activity,' eds. J. F. Griffin and W. L. Duax, Elsevier Biomedical, New York, 1982, pp. 193ff.