Tetrahedron Letters, Vol.32, No.34, pp 4375-4376, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

For-Met-Δ^zLeu-Phe-OMe: A NEW ACTIVE ANALOG OF CHEMOTACTIC N-FORMYLTRIPEPTIDES WITH ß-TURN CRYSTAL CONFORMATION

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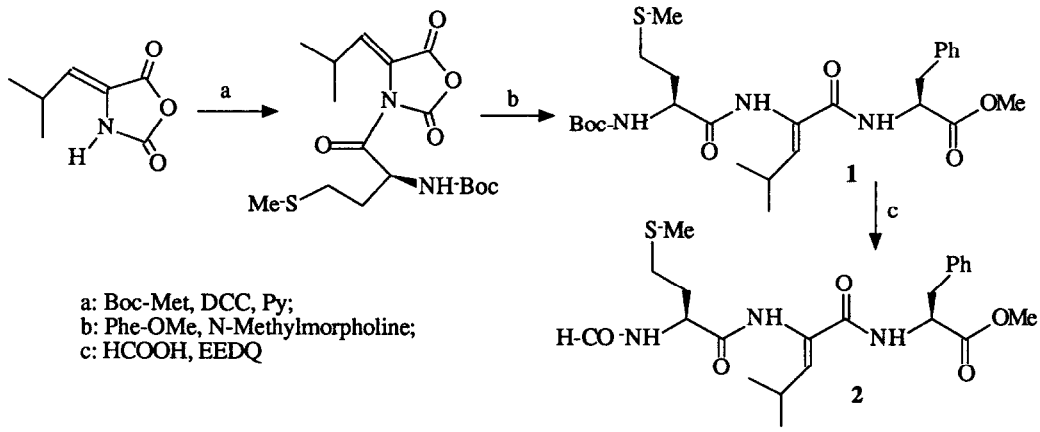
Abstract: *The title Δ^zLeu containing N-formyltripeptide 2, has been synthesized and found active in the superoxide production whereas inactive in the stimulation of human neutrophil migration. The X-ray crystallographic analysis reveals that 2 adopts a β-turn conformation stabilized by an intramolecular H-bond.*

The migration of phagocytic cells (e.g. human neutrophils) directed against invading microrganisms is stimulated by several specific substances termed chemotactic factors[1].

The tripeptide formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) is one of the most potent and structurally simple of these agents. The action of fMLP and related peptides on human neutrophils is mediated by the interaction with specific membrane receptors and includes, in addition to the directed migration, other biochemical events among which release of lysosomal enzymes and production of superoxide anion[1]. Several conformationally restricted analogs of fMLP have been synthesized and examined in order to obtain more potent and selective agonists and to gain information on the features of the receptor bound conformations[2]. Recently For-Met-Leu-Δ^zPhe-OMe, the first fMLP analog containing an α,β-unsaturated residue, has been reported and shown to prefer extended backbone conformation in organic solvent solution and to be highly active[3]. Here we report preliminary information concerning synthesis, conformation in the crystal and activity of a new fMLP analog, *i.e.* For-Met -Δ^zLeu-Phe-OMe (2) designed in order to stabilize a folded conformation by inserting the Δ^zLeu residue at the central position of the peptide backbone.

The intermediate Boc-Met-Δ^zLeu-Phe-OMe (1) was obtained by following the one pot method of Shin[4] starting from *N*-carboxy-(Z)-α,β-didehydroleucine anhydride (Δ^zLeu-NCA); this was treated in sequence with *N*-t-butyloxycarbonyl-L-methionine (Boc-Met)/dicyclohexylcarbodiimide and L-phenylalanine methyl ester (Phe-OMe). Treatment of the *N*-protected tripeptide **1** with formic acid followed by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) gave For-Met-Δ^zLeu-Phe-OMe (2) without affecting the unsaturated residue.



a: Boc-Met, DCC, Py;
b: Phe-OMe, N-Methylmorpholine;
c: HCOOH, EEDQ

The biological activity of **2** has been determined on human neutrophils and compared with that shown by fMLP-OMe . Both directed migration and superoxide anion production have been measured. Whereas **2** is practically inactive in the stimulation of directed migration, it is highly active in the superoxide generation with a potency higher (>50%) than that shown by the parent peptide[5]. The conformational restriction imposed on the backbone by the presence of the α,β-unsaturated residue may be responsible for this selective bioactivity.

In order to examine the conformational preference of **2**, an X-ray crystallographic analysis was undertaken. Suitable single crystals of **2** were obtained from benzene-*n*-hexane; m.p.151-152°C, $[\alpha]_D$ +17° (*c* 1.0, $CHCl_3$). Crystals are monoclinic, space group A2 with a = 5.686(3), b = 16.289(15), c = 32.678(23) Å, β = 96.62(5)°, V = 3006(3) $Å^3$, Z = 4, d_c = 1.12 $g \cdot cm^{-3}$. By using graphite monochromatized Cu-Kα radiation, 2106 reflections with $I > 2.5\ \sigma(I)$ have been collected on an automatic four-circle diffractometer Syntex $P2_1$. The structure has been solved by MULTAN[6] and refined by full-matrix least-squares method to R = 0.11[7]. A perspective view of the peptide conformation found in the crystal is given in the figure. The sequence of backbone torsion angles (see Table) shows that the replacement of the central leucine of fMLP-OMe with the corresponding α,β-unsaturated amino acid, induces a type-II β-turn conformation[8] in which the new residue occupies the i+2 position of the turn. A weak intramolecular H-bond occurs between the formyl C=O and the Phe NH groups with a N··· O contact of 3.15(2) Å . This conformation strikingly differs from that found in the crystal of both the parent tripeptide fMLP-OMe[9] and all the previously studied *N*-formyl derivatives[10,11]. Conformations of the type adopted by **2** have been detected only in solution[12] and in the crystal state of *N*-Boc protected analogs[2]. Thus, the present study reports the first crystal structure revealing a β-turn conformation in a bioactive *N*-formyl peptide.

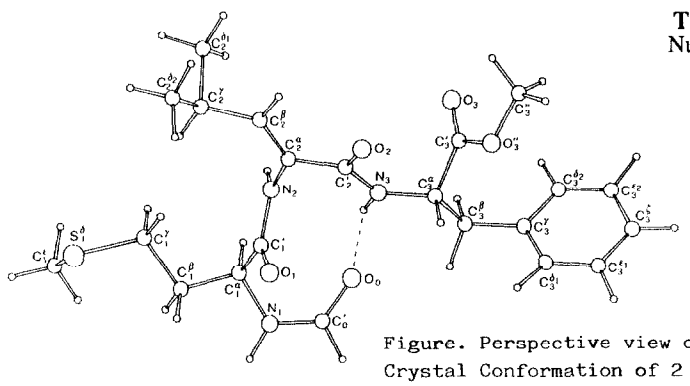


Figure. Perspective view of Crystal Conformation of 2

Table. Backbone torsion angles (°) of **2**. Numbers in parentheses are e.s.d. values

$O_0\text{-}C'_0\text{-}N_1\text{-}C^{\alpha}_1$	(ω_0)	3(1)
$C'_0\text{-}N_1\text{-}C^{\alpha}_1\text{-}C'_1$	(φ_1)	-55(1)
$N_1\text{-}C^{\alpha}_1\text{-}C'_1\text{-}N_2$	(ψ_1)	129.7(8)
$C^{\alpha}_1\text{-}C'_1\text{-}N_2\text{-}C^{\alpha}_2$	(ω_1)	175.0(8)
$C'_1\text{-}N_2\text{-}C^{\alpha}_2\text{-}C'_2$	(φ_2)	72.2(9)
$N_2\text{-}C^{\alpha}_2\text{-}C'_2\text{-}N_3$	(ψ_2)	14.3(8)
$C^{\alpha}_2\text{-}C'_2\text{-}N_3\text{-}C^{\alpha}_3$	(ω_2)	171.5(8)
$C'_2\text{-}N_3\text{-}C^{\alpha}_3\text{-}C'_3$	(φ_3)	-50.4(8)
$N_3\text{-}C^{\alpha}_3\text{-}C'_3\text{-}O''_3$	(ψ_3)	137.2(7)

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(Received in UK 11 May 1991)