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THE ROLE OF PEPTIDE BOND IN CHEMOTACTIC FOR-MET-LEU-PHE-OME FOCUSED BY DEPSIPEPTIDE ANALOGS

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Abstract: The formyl tridepsipeptides HCO-Met-Ψ[COO]Leu-Phe-OMe **3**, HCO-Met-Ψ[COO]Aib-Phe-OMe **4** and HCO-Met-Leu-Ψ[COO]Phe-NHBzl **5** were synthesized in order to investigate the biological consequences arising from introduction of an ester bond at various positions in the chemotactic tripeptides. While analogs **3** and **4** were found lacking in biological activity, stressing an essential role for peptide bond at position 2, compound **5** showed to retain activity.

Chemotactic agents induce directional cell migration along a concentration gradient and are responsible for the accumulation of neutrophils in inflammation sites. Among chemotactic substances, HCO-Met-Leu-Phe-OH (fMLP) is the prototype of small peptides coming from different sources, including bacteria and mast cells[1], which stimulate a wide range of biological functions including chemotaxis, lysosomal enzyme release and oxygen radical generation[2]. Moreover, both fMLP analogs, HCO-Met-Leu-Phe-OMe[3] **1** and HCO-Met-Aib-Phe-OMe **2**, were reported to retain biological activity on human and/or rabbit neutrophils[4].

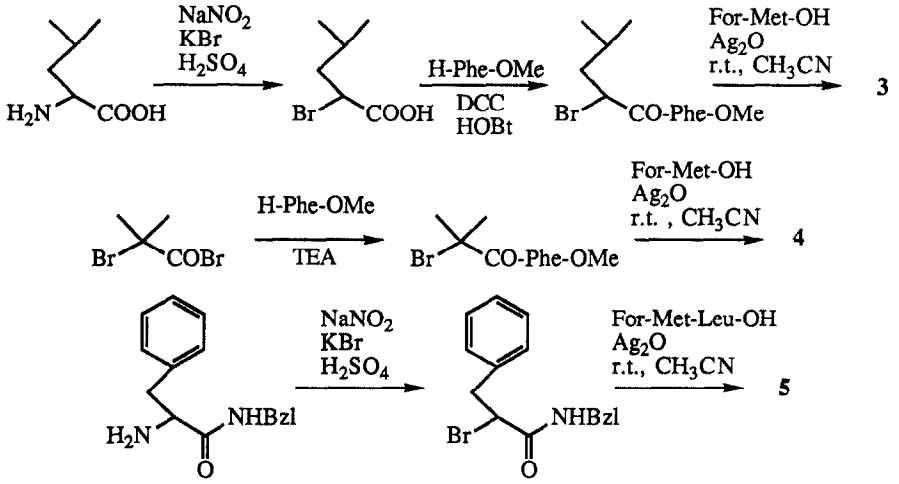
A great number of studies[5] stressed the importance of (a) the formyl group, (b) the methionine at position 1, and (c) the phenylalanine at position 3, which all play a crucial role in both recognition and activation of the chemotactic receptor; the majority of present efforts are aimed at finding substitutions that can improve biological activity. In addition, conformationally restricted tripeptides have been synthesized[6] in which Leu residue at position 2 is substituted with bulky and/or Cα,α dialkylated residues, in order to determine the conformation that can best interact with the specific receptor.

In order to better understand the role played by the peptide bond in the structure-activity relationship, we synthesized the tridepsipeptides HCO-Met-Ψ[COO]Leu-Phe-OMe **3**, HCO-Met-Ψ[COO]Aib-Phe-OMe **4** and HCO-Met-Leu-Ψ[COO]Phe-NHBzl **5**, in which an amide linkage is replaced in two different positions with an ester bond. In the synthesis of **5** we chose the NH-Bzl derivative because only in the presence of a protic amide[8] the condensing agent Ag_2O labilizes the C-Br bond .

The analog **3** of the biologically active compounds **1** as the prototype of classic chemotactic peptides, and the analog **4** of the compound **2** as the prototype of Cα,α dialkylated chemotactic peptides, do not possess the NH group at position 2. As this group in the active compounds does not seem generally involved as proton donor in an intramolecular hydrogen bond and considering the very low chemotactic activity of HCO-Met-Pro-Phe-OMe[7] lacking in amide proton, this could suggest that the introduction of the ester group, which is merely a hydrogen bond acceptor, in compounds **3** and **4** may cause changes in biological activity evidencing the essential role of NH at position 2.

In this paper we describe the synthesis and the results of biological tests, i.e. chemotaxis, lysozyme release and superoxide anion production of compounds **3**, **4** and **5**. The biological data were compared with those of the parent compound **1**.

The ester linkage of compounds **3** and **4** is obtained by coupling the pertinent "bromodipeptides" $BrCH(CH_2CH(CH_3)_2)CO$-PheOMe and $BrC(CH_3)_2CO$-PheOMe with the N-formyl-L-methionine, using condensing agent Ag_2O at room temperature in anhydrous CH_3CN, following a very useful methodology which we set up in our laboratory[8]. Analogous methodology allowed the synthesis of compound **5**.



Formyl tridepsipeptides **3**[9], **4**[10] and **5**[11],were purified by HPLC and characterized by TLC and ^{1}H-NMR. All microanalytical values for C, H and N were satisfactory: H±0.25, N±0.25 and C±1.00.

Surprisingly, as showed below, under these conditions, **3** and **4** were found unable to elicit all the biological responses tested[12] while **5** retains activity.

chemotactic activity

log [M]	-10	-9	-8	-7	-6	-5
HCO-Met-Ψ[COO]Leu-Phe-OMe	0	11	8	13	9	10
HCO-Met-Ψ[COO]Aib-Phe-OMe	10	9	14	14	7	4
HCO-Met-Leu-Ψ[COO]Phe-NHBzl	62	54	51	51	55	20
HCO-Met-Leu-Phe-OMe	105	115	105	85	62	36

Chemotactic activity is expressed as percentage of random locomotion. [The actual control random movement is 32 μm ± 3 Standard Error (SE) of ten separate experiments done in duplicate]. The points are the mean of five separate experiments done in duplicate. SE are in 1-5% range.

superoxide anion release

log [M]	-8	-7	-6	-5
HCO-Met-Ψ[COO]Leu-Phe-OMe	0	0	0	0
HCO-Met-Ψ[COO]Aib-Phe-OMe	0	0	0	0
HCO-Met-Leu-Ψ[COO]Phe-NHBzl	8	14	24	32
HCO-Met-Leu-Phe-OMe	7	44	43	37

Results are expressed as nmoles of superoxide anion/$2x10^6$ neutrophils/5 min,using a 15.5 millimolar extinction coefficient at 550 nm for cytochrome C. The points are the mean of five separate experiments done in duplicate. SE are in 0.5-4% range.

	lysozyme release			
log [M]	-8	-7	-6	-5
HCO-Met-Ψ[COO]Leu-Phe-OMe	11	10	12	10
HCO-Met-Ψ[COO]Aib-Phe-OMe	15	11	13	15
HCO-Met-Leu-Ψ[COO]Phe-NHBzl	24	35	38	40
HCO-Met-Leu-Phe-OMe	54	59	67	62

Results are expressed as percentage of total enzyme content released by 0.1% Triton X-100. The points are the mean of five separate experiments done in duplicate. SE are in 1-6% range.

The above results provide strong evidence that substitution of the peptide bond between residues **1** and **2** with an ester linkage such as in **3** and **4** abolishes all biological activity. Indeed these seemingly negative findings stress the importance and the positive role of the NH group at position 2 in the recognition and/or in an efficient binding with the receptor site. These findings have been further on emphasized by biological behaviour of compound **5** that possesses a biological activity despite the substitution of the amide linkage with an ester bond, clearly showing that the NH group at position 3 of the parent formyltripeptide is not involved, as the NH group at position 2, in a linkage with the biological receptor.

Studies are in progress in our laboratory; the aim is to synthesize further analogs of fMLPOMe, in which the amide bond at position 2 and/or position 3 will be substituted by other non hydrogen bonding groups.

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References and Notes

All the amino acids are in L configuration. No racemisation was found by HPLC analysis.

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9 HCO-Met-Ψ[COO]Leu-Phe-OMe. M.p. 77-79°C; TLC (SiO_2 gel plates 60F-254, Merck) Rf1 (toluene-ethyl acetate 1:4) 0.13; ^{1}H-NMR ($CDCl_3$) δ 8.24 (s, 1H, HCO), 7.29 (s, 5H, phenyl), 6.63(br, 1H, NH), 6.54 (br, 1H, NH), 5.18 (m, 1H, CH), 4.75 - 4.95 (2m, 2H, 2CH), 3.75 (s, 3H, CH_3), 3.23 (ABX, dd, J_{AB}=13.9Hz, J_{AX}=5.6Hz, 1H, CH_2), 3.12 (ABX, dd, J_{AB}=13.9Hz, J_{AX}=6.56Hz, 1H, CH_2), 2.58 (m, 2H, CH_2), 2.19 (s, 3H, CH_3), 2.05 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 0.89 and 0.92 (2d, J=4.0Hz, 6H, $2CH_3$).

10 HCO-Met-Ψ[COO]Aib-Phe-OMe. M.p. 78-81°C; Rf1 0.15; ^{1}H-NMR ($CDCl_3$) δ 8.16 (s, 1H, HCO), 7.29 (s, 5H, phenyl), 6.65 (br, 1H, NH), 6.25 (br, 1H, NH), 4.88 (m, 1H, CH), 4.70 (m, 1H, CH), 3.75 (s, 3H, CH_3), 3.17 (dJ=5.93Hz, 2H, CH_2), 2.56 (t, J=7.25Hz, 2H, CH_2),

11 2.12 (m, 2H, CH_2), 2.10 (s, 3H, CH_3), 1.62 (m, 2H, CH_2), 1.59 and 1.64 (2s, 6H, $2CH_3$).
HCO-Met-Leu-Ψ[COO]Phe-NHBnz. M.p. 129-131°C; Rf1 0.16; ^{1}H-NMR ($CDCl_3$) δ 8.02 (s, 1H, HCO), 7.29 (s, 5H, phenyl), 6.84 (br, 1H, NH), 6.99 (br, 1H, NH), 5.95 (br, 1H, NH), 5.51 (ABX, dd, J_{AX}=4.2Hz 1H, J_{BX}=8.56Hz CH), 4.55-4.66 (m, 3H, CH+1/2CH_2), 4.16-4.28 (m, 3H, CH+1/2CH_2), 3.38 (ABX, dd, J_{AB}=14.41Hz, J_{BX}=4.2Hz, 1H, CH_2), 3.06 (ABX, dd, J_{AB}=14.41Hz, J_{AX}=8.56Hz, 1H, CH_2), 2.43 (t, J=6.84Hz, 2H, CH_2), 2.07 (s, 3H,

12 CH_3), 1.65 (m, 2H, CH_2), 1.40 (m, 2H, CH_2), 0.78 and 0.83 (2d, J=6.0Hz, 6H, $2CH_3$).
Human neutrophils were purified employing the standard techniques of dextran sedimentation of heparinized blood, followed by centrifugation on Ficoll-Paque. The cells were washed twice and resuspended in Krebs-Ringer-phosphate (KRP), pH 7.4, at a concentration of $50x10^6$ cells/ml. Chemotaxis was performed with a 48-well microchemotaxis chamber, and the migration into the filter was evaluated by the leading-front method[a]. The dose-response curves are typical of chemoattractants that rise to a peak and then decline to zero as the concentration of ligand is increased above its optimum value[4c]. A likely explanation for this decrease is that the chemical gradient was diminished from the increase rate of diffusion of that attractant, resulting in a greater degree of saturation of the cells in the upper compartment of the chemotactic chamber[4e]. Superoxide anion release was monitored continuously in a thermostated spectrophotometer as superoxide reduction of ferricytochrome c[b]. Release of neutrophil granule lysozyme was quantified nephelometrically by the rate of lysis of cell wall suspension of Micrococcus lysodeikticus[b]. Stock solution of each peptide (10^{-2} M in dimethylsulfoxide) was diluted before use in KRP at final concentrations of 10^{-10}-10^{-5} M.

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