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Synthesis of Peptides Containing Bis-Imidazole Ligands

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Abstract *- The coupling of bis[imidazol-2-yl]methylamine to the carboxyl terminus of peptides and of 3-[bis(BOC-imidazol-2-yl)]propionic acid to the amino terminus of peptides is described.*

The imidazole ring of histidine plays an important role in the metal binding site of zinc proteases. For example, in carboxypeptidase A[1] zinc is coordinated by two imidazole rings and a carboxylate anion, in carbonic anhydrase[2] and in collagenase[3] by three imidazole rings. To mimic the binding and catalytic activities of some of these enzymes, the synthesis of polyimidazole ligands was developed.[4] Such ligands may also serve as general zinc enzyme inhibitors. Moreover, specific inhibitors may be obtained by attaching these ligands to the preferred peptide sequence for the enzyme cleavage.

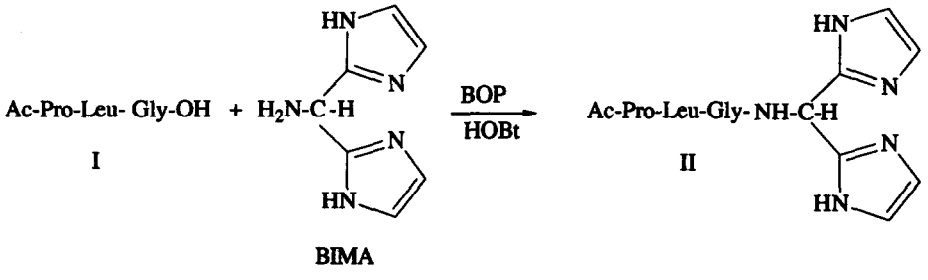
We now wish to report on the synthesis of peptides containing imidazole ligands either on their N- or on their C-termini. For the illustration of these procedures we describe below the coupling of bis-imidazole containing agents such as bis[imidazol-2-yl]methylamine[5] (BIMA) and 3-[bis(imidazol-2-yl)]propionic acid[5] (BIP) to the two fragments of the specific sequence -Pro-Leu-Gly-Ile-Ala-Gly- cleaved by vertebrate collagenases at the Gly-Ile bond.

For the synthesis of the free carboxyl group containing fragment I, equivalent amounts of H-Leu-Gly-OMe×HCl[6] and Ac-Pro-OH were coupled at 0°C in DMF[7] in the presence of equivalent amounts of TEA and DCC. Next day DCU was filtered off, the filtrate was evaporated in vacuo and the residue was purified on silica gel column in solvent system A[8] (R_f 0.68) to give Ac-Pro-Leu-Gly-OMe with 65% yield. Then the tripeptide ester was saponified with two equivalents of N NaOH solution and evaporated in

vacuo. The residue was dissolved in abs. ethanol and the inorganic salts were filtered off. The filtrate was concentrated and the residue triturated with ether to give I (R_f 0.35, A) in 73% yield.[9]

The coupling of BIMA×3HCl to the acetyl tripeptide (Scheme I) failed by the usual DCC condensing method, but it was successful when using the BOP[10] reagent as follows. The solution of I (1.0 g, 3.05 mmole), DIEA (0.52 ml, 3.05 mmole), BOP (1.49 g, 3.4 mmol) and HOBt (0.45 g, 3.4 mmole) in DMF (5 ml) was stirred for 10 min, then poured into the solution of BIMA×3HCl (0.83 g, 3.05 mmol) and DIEA (1.57 ml, 9.15 mmole). The pH of the solution was adjusted to 8 with some drops of DIEA, and the reaction mixture was stirred at room temperature for 8 h. The precipitate was filtered off and the filtrate was evaporated in vacuo. The residue was purified on a silica gel column with eluent A (R_f 0.58) yielding 680 mg (47%) II. Further purification was carried out by RP-HPLC (Eluent MeOH : 0.06% TFA/water 1 : 1). $C_{22}H_{32}N_8O_4$ (472), MS (EI) observed M 472. ^{1}HNMR: (DMSO-d_6) δ 5.4 (broad signal imidazole N-H), 6.26 (d, 1H, J = 8.3 Hz), 6.92 (s, 4H), 8.35 (d, J = 8.3 Hz, amide N-H).

SCHEME I



I

BIMA

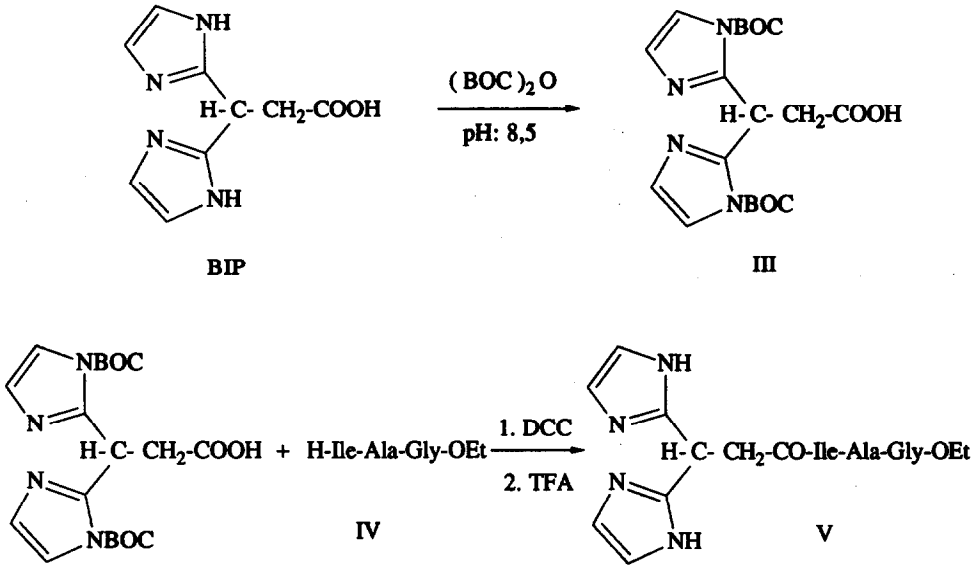
II

The direct coupling of BIP to the amino terminus of peptides did not succeed because of solubility difficulties. Therefore we have prepared the bis-BOC derivative III from BIP with some modification of the method described for the preparation of $N^{\alpha}N^{im}$-bis-BOC-histidine[11] (Scheme II). BIP (5.78 g, 27 mmole) was dissolved in a mixture of water and dioxane (1:1, 60 ml), with the pH of the solution kept at 8.5 during the reaction while the solution of $(BOC)_2O$ (8.81 g, 5 ml dioxane) was added dropwise under stirring. Stirring was continued till no more starting material was detected on TLC. Dioxane was evaporated in vacuo, and the remaining solution was extracted with ether, then acidified with cold citric acid solution, and extracted with ether and

ethylacetate. The two organic layers were combined, washed with water, dried (Na_2SO_4), filtered and evaporated in vacuo to give 6.47 g (59%) III.

For the synthesis of the free amino group containing fragment IV, the solution of H-Ala-Gly-OEt×HCl[12] (2g, 9.48 mmole), NMM (1.05 ml, 9.48 mmole) and BOC-Ile-OSu (3.12 g, 9.48 mmole) in DMF (20 ml) was stirred for 2 hours. The solvent was evaporated in vacuo, the residue triturated with saturated $NaHCO_3$ solution, filtered and crystallized from ethanol-water to give 3.68 g (80%) BOC-Ile-Ala-Gly-OEt (R_f 0.77, B, m.p. 151-152°C). The BOC group was removed by 4N HCl/EtOAc yielding 97% IV×HCl (R_f 0.32 A, 0.57 C).

SCHEME II



The bis-BOC-BIP was coupled to IV with similar efficacy either by using DCC or BOP reagent as described above. In the working up process the reaction mixture was concentrated in vacuo, the residue was dissolved in ethylacetate, washed with cold citric acid solution, water, saturated $NaHCO_3$ solution, then again water. The ethylacetate phase was dried (Na_2SO_4) and evaporated to give 3-[bis(BOC-imidazol-2-yl)]-propionyl-Ile-Ala-Gly-OEt with 85% yield (R_f 0.32 B, 0.87 D). This crude product contained an impurity with R_f 0.77 (B) which corresponds to the R_f of BOC-Ile-Ala-Gly-OEt, indicat-

ing some transfer of the BOC group to the free amino group of the peptide component. For analysis, a sample was dissolved in ethylacetate and precipitated with petrolether. The BOC groups of the crude product were split by TFA and the resulting V was purified on silica gel column in eluent A (R_f 0.55) with 85% yield. $C_{22}H_{33}N_7O_5$ (475.54), MS (EI) observed M 475, (CI) observed M + H^+ 476.

References and Notes

1.) Lipscomb, W.N. *Acc. Chem. Res.* **1970**, 3, 81-89.

2.) Wyeth, P. and Prince, R.H. *Inorg. Perspect. Biol. Med.* **1977**, 1, 37-71.

3.) Woessner, I.F.Jr. *The FASEB Journal*, **1991**, 5, 2145-2154.

4.) Breslow, R., Hunt, J.T., Smiley, R. and Tarnowski, T. *J. Am. Chem. Soc.* **1983**, 105, 5337-42. Mullier, E. *Tetrahedron Lett.* **1989**, 30, 6169-72. Choi, Y.-D. and Street, J.P. *J. Org. Chem.* **1992**, 57, 1258-62, and references therein.

5.) Leigh, M.J. and Swain, M.C. *Synthesis*, **1977**, 459-61.

6.) Pettit, G.R. and Jones, W.R. *J. Org. Chem.* **1971**, 36, 870-73.

7.) Abbreviations: DMF: dimethylformamide, TEA: triethylamine, DCC: dicyclohexylcarbodiimide, DCU: dicyclohexylurea, BOP: (benzotriazolyloxy)tris(dimethylamino)-phosphonium hexafluorophosphate, HOBt: 1-hydroxybenzotriazole, DIEA: diisopropylethylamine, BOC: tert-butyloxycarbonyl, TFA: trifluoroacetic acid, $(BOC)_2O$: di-tert-butyldicarbonate.

8.) Solvent systems for silica gel chromatography: A (ethylacetate : pyridine : acetic acid : water = 60 : 20 : 6 : 11), B (ethylacetate : pyridine : acetic acid : water = 480 : 20 : 6 : 11), C (butanol : acetic acid : water = 4 : 1 : 1), D (butanol : pyridine : acetic acid : water = 4 : 1 : 1 : 1).

9.) Analytical data for the newly synthesized compounds were satisfactory.

10.) Castro, B., Dormoy, J.R., Evin, G., Selve, C. *Tetrahedron Lett.* **1975**, 14, 1219-22.

11.) Schnabel, E., Stoltefuss, J., Offe, H.A., and Klauke, E. *Liebigs Ann. Chem.* **1971**, 743, 57-68.

12.) Zeiger, A.R., Lange, A. and Maurer, P.H. *Biopolymers* **1973**, 12, 2135-49.

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