# Synthesis of Oligopeptides having Alternate L-Leucyl and L-Methionyl Residues

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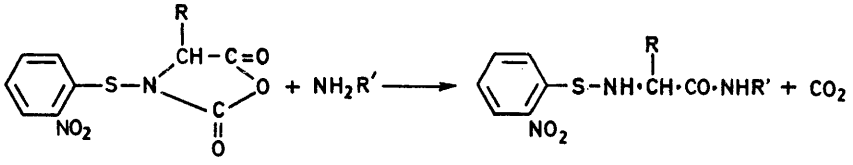
A series of oligopeptides having alternate L-leucyl and L-methionyl residues which are interesting from a conformational aspect have been prepared by a method involving use of an *N*-*o*-nitrophenylsulphenyl-*N*-carboxy-α-amino-acid anhydride (Nps-NCA). The peptides were assembled stepwise, with L-methionine ethyl ester as the starting amino-acid; L-leucyl residues were introduced in high yields by the 'Nps-NCA' method, and L-methionyl residues in moderate yields by the dicyclohexylcarbodi-imide method. The usefulness of the Nps-NCA method for peptide synthesis is demonstrated.

We have demonstrated that *N*-carboxy-α-amino-acid anhydrides (NCAs) protected by the *o*-nitrophenylsulphenyl (Nps)[1] group are very useful for stepwise synthesis of *N*-protected peptides.[2,3] The advantages of this new method result from the use of highly purified, crystalline Nps-NCAs having very high reactivity towards an amino-group, and the absence of by-products (Scheme 1), leading to easy purification and a high yield of the desired peptide. The usefulness of the lack of by-products is enhanced in the preparation of large peptides having low solubility in solvents normally used for purification. We describe here the application of the method to the synthesis of a series of oligopeptides having alternate L-leucyl and L-methionyl residues, which are interesting from a conformational aspect.[4-6]



SCHEME 1

The starting material was L-methionine ethyl ester hydrochloride. L-Leucyl residues were introduced by reaction with Nps-L-leucine NCA and L-methionyl residues by reaction with Nps-L-methionine, with dicyclohexylcarbodi-imide as condensing agent. Treatment of L-methionine ethyl ester with Nps-L-leucine NCA for 2 h at room temperature gave Nps-L-leucyl-L-methionine ethyl ester (1) in 86% yield after recrystallization from ethyl acetate. The Nps-dipeptide ester was treated with 2N-hydrochloric acid in dioxan to remove the protecting group. The resulting dipeptide ester hydrochloride was treated with Nps-L-methionine in the presence of triethylamine and dicyclohexylcarbodi-imide (DCC) for 24 h at 0 °C. After removal of dicyclohexylurea and recrystallization from ethyl acetate, Nps-L-methionyl-L-leucyl-L-methionine ethyl ester (2) was obtained in 82% yield. The peptide chain was further elongated stepwise by removal of the Nps group with hydrochloric acid, followed by alternate reactions, with Nps-L leucine NCA and with Nps-L-methionine and dicyclohexylcarbodi-imide. The synthetic route is summarized in Scheme 2. For the syntheses of the tetrapeptide and lower peptides, tetrahydrofuran was used as reaction solvent. A mixture of tetrahydrofuran and *NN*-dimethylformamide was used for the syntheses of the pentapeptide and higher peptides to improve the solubility of both the reactant peptide ester hydrochloride and the resulting Nps-peptide ester. After every coupling reaction, the system was repeatedly washed with citric acid and aqueous sodium hydrogen carbonate to remove any unchanged reactants, and the product was isolated and purified by recrystallization. Nps-heptapeptide and Nps-octapeptide esters were further purified by dry-

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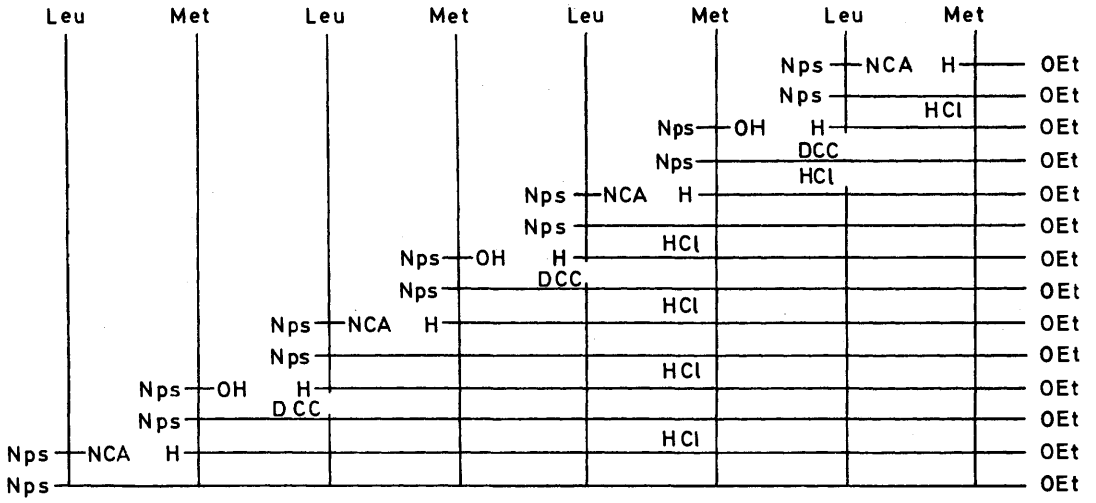
column chromatography on silica gel.[7] Every Nps-peptide ester gave a single spot on t.l.c. (silica gel).

Results of the syntheses are shown in the Table. In the syntheses of the di-, tri-, and tetra-peptides, the Nps-NCA and the dicyclohexylcarbodi-imide methods gave considerations. The lower oligopeptides are very soluble in solvents such as ethyl acetate used for recrystallization, and any insoluble by-product, *e.g.* dicyclohexylurea in the carbodi-imide method, is easily removed by recrystallization. However the higher oligopeptides are of




SCHEME 2

similar yields. However differences in the yields from the two methods were found in the syntheses of higher oligopeptides. The hexapeptide (5) and octapeptide (7), prepared by the Nps-NCA method, were obtained in 77 lower solubility, and repeated recrystallization or chromatography becomes necessary to purify the product, leading to a decrease in yield. For example, the Nps-hexapeptide (5), prepared by the Nps-NCA method, was

Syntheses of Nps-oligopeptide ethyl esters

| Peptide [a] | Yield (%) | M.p. (°C) | $[\alpha]_D$ (°) | $R_F$ (t.l.c.) [d] | Found (%) C | Found (%) H | Found (%) N | Required (%) C | Required (%) H | Required (%) N |
|---|---|---|---|---|---|---|---|---|---|---|
| (1) | 86 | 92—93 | −57.1 [b] | 0.85 [e] | 51.6 | 6.6 | 9.5 | 51.5 | 6.6 | 9.5 |
| (2) | 82 | 134—135 | −37.2 [b] | 0.76 [e] | 50.1 | 6.6 | 9.8 | 50.2 | 6.7 | 9.8 |
| (3) | 76 | 202—203 | −63.9 [b] | 0.79 [f] | 52.3 | 7.2 | 10.3 | 52.4 | 7.2 | 10.2 |
| (4) | 52 | 239—241 | −48.4 [b] | 0.81 [f] | 51.3 | 7.2 | 10.2 | 51.3 | 7.1 | 10.3 |
| (5) | 77 | 250—251 | −59.0 [b] | 0.92 [g] | 52.9 | 7.5 | 10.5 | 52.8 | 7.5 | 10.5 |
| (6) | 52 | 267—270 † | −28.5 [c] | 0.78 [h] | 52.1 | 7.4 | 10.5 | 52.0 | 7.4 | 10.5 |
| (7) | 63 | 275—280 † | −41.2 [c] | 0.85 [i] | 53.1 | 7.7 | 10.7 | 53.1 | 7.6 | 10.7 |

[a] All amino-acid residues have the L-configuration; the compounds are numbered as follows: (1) Nps-Leu-Met-OEt, (2) Nps-Met-Leu-Met-OEt, (3) Nps-Leu-Met-Leu-Met-OEt, (4) Nps-Met-Leu-Met-Leu-Met-OEt, (5) Nps-Leu-Met-Leu-Met-Leu-Met-OEt, (6) Nps-Met-Leu-Met-Leu-Met-Leu-Met-OEt, (7) Nps-Leu-Met-Leu-Met-Leu-Met-Leu-Met-OEt. [b] (*c* 1.0 in tetrahydrofuran). [c] (*c* 1.0 in *NN*-dimethylformamide). [d] Developing solvents: [e] ethyl acetate–benzene (1 : 1); [f] tetrahydrofuran–benzene (1 : 1); [g] tetrahydrofuran–benzene–methanol (10 : 10 : 1); [h] tetrahydrofuran–*NN*-dimethylformamide (4 : 1); [i] tetrahydrofuran–*NN*-dimethylformamide (3 : 1).

† Decomp.

and 63% yields, respectively, but the pentapeptide (4) and heptapeptide (6), prepared by the carbodi-imide method were isolated in 52% yield. Since these yields refer to different stages in the synthesis of the peptide, *i.e.* L-leucyl residues are coupled by the Nps-NCA method and L-methionyl residues by the carbodi-imide method, they are not strictly comparable. Nevertheless the Nps-NCA method appears to give somewhat higher yields than the carbodi-imide method in the syntheses of higher oligopeptides. This trend may be explained by solubility easily purified two recrystallizations from tetrahydrofuran, but the lower peptide (4), obtained by the carbodi-imide method, still contained a small amount of dicyclohexylurea after three recrystallizations from the same solvent. Thus the higher yields in the Nps-NCA method may result not only from the higher reactivity of the NCA derivative,[8-11] but also from the lack of by-products, other than carbon dioxide.

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EXPERIMENTAL

L-Methionine ethyl ester hydrochloride (30.8 g) suspended in tetrahydrofuran (300 ml) was treated with triethylamine (22.8 ml). The solution was treated with Nps-L-leucine NCA (42.2 g) by the general procedure [2] to give Nps-L-leucyl-L-methionine ethyl ester (51.0 g, 84%). The Nps group of the dipeptide derivative (1) (35.5 g) was removed by dissolution in 2N-hydrochloric acid in dioxan (80 ml). The resulting dipeptide ester hydrochloride (25.7 g) was treated with Nps-L-methionine dicyclohexylammonium salt [12] (38.2 g) in the presence of dicyclohexylcarbodi-imide (18.0 g) in tetrahydrofuran (400 ml). Nps-L-methionyl-L-leucyl-L-methionine ethyl ester (2) was obtained in 82% yield after recrystallization from ethyl acetate. Nps-L-leucyl-L-methionyl-L-leucyl-L-methionine ethyl ester (3) was obtained by the reaction of Nps-L-leucine NCA with L-methionyl-L-leucyl-L-methionine ethyl ester hydrochloride, prepared by removal of the Nps group from (2) with hydrochloric acid, in 76% yield after two recrystallizations from ethyl acetate–tetrahydrofuran (2 : 1). Removal of the Nps group from (3), followed by reaction with Nps-L-methionine dicyclohexylammonium salt in tetrahydrofuran *NN*-dimethylformamide (5 : 1) gave Nps-L-methionyl-L-leucyl-L-methionyl-L-leucyl-L-methionine ethyl ester (4) in 52% yield after four recrystallizations from warm tetrahydrofuran. The Nps-hexapeptide ethyl ester (5) was prepared by the Nps-NCA method in tetrahydrofuran in 77% yield after two recrystallizations from tetrahydrofuran. Further reaction with Nps-L-methionine dicyclohexylammonium salt in the presence of dicyclohexylcarbodi-imide gave Nps-L-methionyl-L-leucyl-L-methionyl-L-leucyl-L-methionyl-L-leucyl-L-methionine ethyl ester (6) in 52% yield after three recrystallizations from *NN*-dimethylformamide, followed by dry column chromatography on silica gel (10 × 10 cm) with ethyl acetate–tetrahydrofuran (1 : 1). The final product, Nps-L-leucyl-L-methionyl-L-leucyl-L-methionyl-L-leucyl-L-methionyl-L-leucyl-L-methionine ethyl ester (7) was prepared from (6) with Nps-L-leucine NCA in tetrahydrofuran–*NN*-dimethylformamide (1 : 2) in 63% yield after dry column chromatography and recrystallization from *NN*-dimethylformamide.

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