CHEMISTRY LETTERS, pp. 1529-1530, 1974. Published by the Chemical Society of Japan

A NEW METHOD FOR PEPTIDE SYNTHESIS USING o-NITROPHENYLSULFENYL N-CARBOXY α-AMINO ACID ANHYDRIDES

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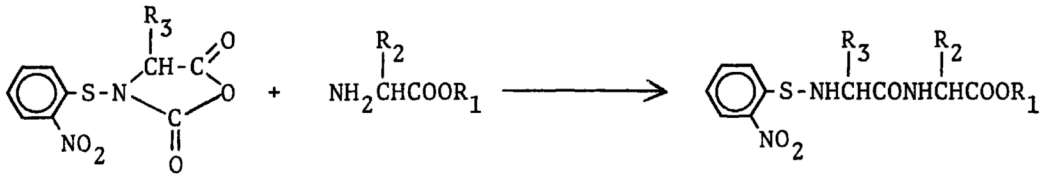
A New method for peptide synthesis using o-nitrophenylsulfenyl α-amino acid anhydrides (Nps-NCAs) is reported. The method involves the reaction of the Nps-NCA with an amino acid ester or a peptide ester to give a N-protected peptide ester by the Nps group. Some peptide derivatives have been easily prepared without racemization in very high yields by the new method. The advantages of this method may result from the rapid acylation of the amino group by the NCAs without formation of by-product.

We wish to report a new method for peptide synthesis involving stepwise synthesis of peptide chains by using o-nitrophenylsulfenyl (Nps) N-carboxy α-amino acid anhydrides (NCAs).[1] Our method has two characteristic advantages resulting from the use of the Nps-NCAs. The first is rapid synthesis of peptide chains. The highest reactivity of the anhydride group of the NCAs which has been demonstrated by many studies of polymerization[2] can accomplish almost quantitatively the acylation of the amino group of amino acids and peptides in a few hours at room temperature. The second is that the reaction of the Nps-NCAs gives no by-product so that the desirous peptide may be easily purified to lead to a high yield. The only by-product accompanied by the reaction is carbon dioxide which leaves as a gas from the reaction system. The yield of peptides is also improved by the use of highly purified NCAs, which differs from the peptide synthesis by conventional mixed anhydride method[3] using impure anhydrides.

We prepared Nps-L-Asp(OBzl)-L-Leu-OBzl in 98% yield by the Nps-NCA method. L-Leucine benzyl ester, 0.02 mol was treated with Nps-β-benzyl L-aspartate NCA, 0.02 mol in 200 ml of tetrahydrofuran (THF) or acetonitrile for 2 hours with stirring. After the reaction, the solution was concentrated to give an oil, which was dissolved in ethyl acetate. The solution was washed with aqueous solution of sodium bicarbonate and of citric acid, and water. The dried solution was concentrated at reduced pressure. The residual oil was crystallized by addition of n-hexane. The product was recrystallized from ethyl acetate. 11.3 g (98%). mp 112-113°C $[\alpha]^{25}_{D}$ -25.6° (c 1.0,THF) Anal. Calcd for $C_{30}H_{33}N_3O_7S$: C,62.16; H,5.74; N,7.25. Found: C,62.08; H,5.80; N, 7.23.

Another dipeptide, Nps-L-Val-L-Ala-OBzl was synthesized analogously in 88% yield. The physical properties of the sample obtained by the Nps-NCA method were completely consistent with those of an authentic sample prepared by dicyclohexylcarbodiimide

method. The dipeptide prepared by our method showed: mp 134.5-135.5°C $[\alpha]^{25}_{D}$ -88.15° (c 2.0, ethyl acetate), -103.1° (c 1.0, THF). Anal. Calcd for $C_{21}H_{25}N_3O_5S$: C,58.46; H,5.84; N,9.47. Found: C,58.42; H,5.91; N,9.50. The authentic sample showed: mp 134.5-135.5°C $[\alpha]^{25}_{D}$ -88.15° (c 2.0, ethylacetate), -102.9° (c 1.0,THF). These results show that the new method is practically free from racemization during the formation of peptide bond.



We tried to elongate the peptide chains by the Nps-NCA method. The Nps-protecting group of Nps-L-Asp(OBzl)-L-Leu-OBzl was removed by action of 1.6N-hydrochloric acid in dioxane. The resulting dipeptide ester hydrochloride was allowed to react with Nps-L-Lys(Z) NCA in the same manner as above to give Nps-L-Lys(Z)-L-Asp(OBzl)-L-Leu-OBzl in 84% yield after recrystallization from ethyl acetate. mp 128-130°C $[\alpha]^{30}_{D}$ -16.4° (c 1.0, DMF) Anal. Calcd for $C_{44}H_{51}N_5O_{10}S$: C,62.62; H,6.09; N,8.54. Found: C,62.48; H,6.15; N,8.39.

An example for preparing protected tripeptide was also given by the synthesis of Nps-L-Glu(OBzl)-L-Val-L-Ala-OBzl. Nps-L-Glu(OBzl) NCA was allowed to react with HCl·H-L-Val-L-Ala-OBzl prepared by the Nps-NCA method. After treatments for isolation and purification, the product was obtained in 82% yield. mp 165-167°C $[\alpha]^{30}_{D}$ -11.0 (c 1.0, DMF) Anal. Calcd for $C_{33}H_{38}N_4O_8S$: C,60.91; H,5.89; N,8.61. Found: C,60.98; H,5.92; N,8.58.

The chain lenthening of peptides by the Nps-NCA method was successfully extended to a tetrapeptide derivative, Nps-L-Val-L-Phe-L-Lys(Z)-L-Ala-OBzl. The peptide chain was elongated from L-alanine benzyl ester by sequential treatments with Nps derivative of L-Lys(Z) NCA, L-Phe NCA, and L-Val NCA including purification of interemediate peptide derivatives and removal of the Nps-protecting group. The tetrapeptide was obtained in 70% yield from L-alanine benzyl ester. mp 190-192°C $[\alpha]^{30}_{D}$ -13.8° (c 1.0, DMF) Anal. Calcd for $C_{44}H_{52}N_6O_9S$: C,62.84; H,6.23; N,9.99. Found: C,62.75; H,6.30; N,9.85. Two hours was adequate reaction time to accomplish the elongation of each peptide bond. The pure interemediates were obtained by once recrystallization.

The facile synthesis of these peptide derivatives described here may result from the rapid acylation of the amino group by the NCAs without formation of by-product leading to easy purification of the peptides. We believe that the new method is very useful for the stepwise synthesis of the protedted peptide by Nps group. The application of the new method for the synthesis of higher and biologically active peptides is now under investigation.

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(Received October 26, 1974)