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A SHORT-CUT IN PEPTIDE SYNTHESIS

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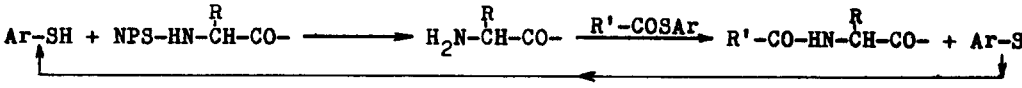
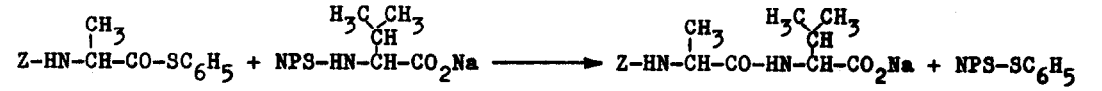
One step in peptide synthesis involves the selective removal of an amino-protecting group from a fully protected peptide, in order to make available the amino function for further peptide bond formation with an N-protected amino acid or peptide. The simultaneous removal of an N-protecting group and peptide bond synthesis could be achieved, in principle, if the coupling reaction generated an agent capable of selective removal of the N-protecting group. Such a condition is met when aryl thiolesters (1) of N-protected amino acids are used as the "carboxyl activated component" during peptide bond synthesis with o-nitrophenylsulfenyl (NPS) amino acids or peptides (2)([X]). During such a reaction, the aminolysis of the thiolester produces the corresponding thiol, an agent capable of selective removal of the NPS group (3):



While the reaction course could be as above, several experiments have shown that aryl thiolesters react with the sodium salts of NPS-amino acids or peptides without added free thiol or free amine:



with Z- = $C_6H_5-CH_2-O-CO-$; NPS- = $o-NO_2-C_6H_4-S-$

([X]) While these experiments were under way, we learned from Dr. Y.V. Mitin that he has obtained some related results (in press). Several aspects of the chemistry of aryl thiolesters and of arylsulfenylamides are described in: Y.V. Mitin and G.P. Vlasov, Dokl. Akad. Nauk S.S.S.R., 1968, 179, 353.

In a typical example which serves to outline the experimental details, Z-glycine phenyl thiolester (5mM) and NPS-L-alanine (5mM) are dissolved in tetrahydrofuran (16ml) and methanol (6ml) in the presence of N NaOH (5ml). The reaction mixture is heated at 70° for 6 hours. After evaporation and addition of dilute aqueous $NaHCO_3$, the alkaline solution is extracted with ethyl acetate and acidified with 5N H_2SO_4. The reaction product is extracted with ethyl acetate and, after washing with water and drying over $MgSO_4$, the solution is evaporated to dryness. Z-glycyl-L-alanine (1,4) crystallises in 62% yield from ether (m.p. 131-133°; $[\alpha]_D^{20}$ -10,4°, c 2,5 in ethanol; Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C 55,71 H 5,75 N 9,99; Found: C 55,33 H 5,69 N 9,95). A compound with the same physical constants was obtained in 68% yield when, in otherwise identical reaction conditions, Z-glycine phenyl thiolester was coupled with free alanine.

The reaction conditions are those described in literature for phenyl thiolesters aminolysis (1) and little attempt has been made to improve the product yield. Better yields have however been obtained when a two-fold excess of the phenyl thiolester was used. In this way Z-glycyl-L-alanyl-L-phenylalanine (yield 56%; m.p. 159-163°; $[\alpha]_D^{24}$ -7,3°, c 2,1 in ethanol; Anal. Calcd. for $C_{22}H_{25}N_3O_6$: C 61,82 H 5,89 N 9,83; Found: C 61,67 H 6,02 N 9,80) and Z-L-alanyl-L--alanyl-L-phenylalanine (yield: 74%; m.p. 187-191°; $[\alpha]_D^{21}$ -13,9°, c 2,0 in ethanol; Anal. Calcd. for $C_{23}H_{27}N_3O_6$: C 62,58 H 6,16 N 9,52; Found: C 62,30 H 6,36 N 9,50) were prepared from NPS-L-alanyl-L-phenylalanine and the corresponding Z-amino acid phenyl thiolester. Similarly, reaction of NPS-L-valine with Z-L-alanine phenyl thiolester gave Z-L-alanyl-L-valine in 76% yield (m.p. 146-150°; $[\alpha]_D^{22}$ -16,6°, c 2,0 in ethanol; Anal. Calcd. for $C_{16}H_{22}N_2O_5$: C 59,60 H 6,87 N 8,70; Found: C 59,41 H 7,11 N 8,61).

An attempt to use an NPS-amino acid ethylester for coupling with a phenyl thiolester instead of the corresponding sodium salt, was unsuccessfull. Work is under way to determine the scope and limitations of the above described method. It is certain that this "short-cut" will not eliminate the disadvantages inherent in the NPS group and thiolesters.

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