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PEPTIDE SYNTHESIS USING TRIPHENYL PHOSPHITE AND IMIDAZOLE

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Recently we reported on the possibility of peptide synthesis with triaryl phosphites without the isolation of active esters [1]. This method permits the synthesis of peptides in one step from acyl amino acids and amino acid esters. However, the comparatively small yields, high temperature of reaction and shortcomings characteristic of the active ester method did not allow its use for obtaining a number of peptides. The present communication describes a new modification of the phosphite method based on the use of imidazole.

The addition of imidazole permits the lowering of reaction temperature, gives a significant increase in peptide yield and allows the use of our method for peptide synthesis proceeding from acyl amino acids such as Z-L-Asn, Z-L-Gln and α-Z-ω-NO_2-L-Arg. Peptide synthesis can be carried out in any suitable solvent except chlorinated hydrocarbons (chloroform, methylene chloride etc.) The most suitable solvents are dimethylformamide and dioxane. Concerning the N-protective groups, the best results were obtained using carbobenzoxy- and tert.-butyloxycarbonyl groups. Aryl sulphonyl protection and protection based on β-diketones cannot be tolerated as they interact with phosphites. As to the protection of the carboxyl function, the usual means (esterification) are quite suitable.

The reaction of peptide synthesis follows the scheme:

$$Z\text{-}NHCHR'COOH + NH_2CHR''COOR + P(OC_6H_5)_3 \xrightarrow{\text{imidazole}}$$

$$ZNHCHR'CONHCHR''COOR + (C_6H_5O)_2POH + C_6H_5OH$$

where Z is the carbobenzoxy- or tert.-butyloxycarbonyl group and R is CH_3-, C_2H_5-, $PhCH_2$-, p-nitrobenzyl-.

Besides peptide, diphenyl phosphite and phenol are obtained as a result of the reaction and are easily removed by washing with water and ether. It is expedient to choose the R peptide group in such a way that the formed peptide will not dissolve in the ether; for this purpose the p-nitrobenzyl group is the most suitable. This is especially important when obtaining dipeptides; as for tri-, tetrapeptides etc., the choice of protective groups in this sense is of no special importance as such protected peptides, as a rule, do not dissolve in ether.

For synthesis Z-L-Asn-Gly-OBzlNO$_2$ 0.67 g (2.5 mM) Z-L-Asn, 0.73 g (2.5 mM) HBr·Gly-OBzlNO$_2$ is dissolved in 3 ml of dimethylformamide, 0.34 ml (2.5 mM) Et_3N, 0.26 g (3.8 mM) of imidazole and 0.105 g (3.8 mM) $P(OPh)_3$ are added, and the mixture kept at 40°C for 18 hrs. After usual treatment and washing with ether Z-L-Asn-Gly-OBzlNO$_2$ is obtained with a 96% yield (see Table 1). It is shown by infrared spectroscopy that CN-groups are completely absent even in the unrecrystalized product. Other peptides are synthesized analogously (see Table 1).

For serine and threonine peptide synthesis it is necessary to protect the hydroxyl group.

In order to determine the degree of racemization tripeptide Z-Gly-L-Phe-Gly-OEt was synthesized (Anderson test [2]). The amount of the racemized product formed is less than 0.5%.

The mechanism of reaction, on which the method is based, will be discussed in a separate communication.

TABLE 1

Peptides obtained using triphenylphosphite and imidazole at 40°C for 18 hrs

Peptide	Yield %	Melting point °C	$[\alpha]_D^{23}$
Z-Gly-Gly-OBzlNO$_2$	96	109 (107-108 [3])	
Z-L-Leu-Gly-OBzlNO$_2$*	99	95.5-96.5	-24 (C2, dioxane)
Z-L-Ala-L-Ala-OBzlNO$_2$*	99	139-140	-34 (C2, tetrahydrofuran)
Z-L-Val-Gly-OBzlNO$_2$*	92	160-161	-29.2 (C2, dioxane)
Z-L-Pro-Gly-Gly-OEt (from Z-L-Pro)	90	119.5-120.5 (120 [4])	-26 (C1, ethanol)
Z-Gly-L-Phe-Gly-OEt (from Z-Gly-L-Phe)	97	119-119.5 (119 [2])	-12.4 (C2, ethanol)
Z-L-Asn-Gly-OBzlNO$_2$*	96	198-199	-6.1 (C2, dimethylformamide)
Z-L-Gln-Gly-OBzlNO$_2$*	95	201-202	-5.8 (C2, dimethylformamide)
Z-L-His-L-Ala-OBzlNO$_2$*	93	187.5-188	-14 (C2, dimethylformamide)
Z-L-Arg(NO$_2$)-L-Phe-OEt	92	139-141 (141-143 [5])	+6.5 (C2, acetic acid)
Z$_2$-L-Lys-Gly-OBzlNO$_2$	85	109-110 (94 [3])	-10.7 (C2, acetic acid)
Z-L-Cys(Bzl)-Gly-OEt	96	98 (98-99 [6])	-27.3 (C2, acetic acid)
Z-L-Try-Gly-OMe	97	157-158 (158-159 [7])	-12.7 (C2, acetic acid)

* The results of elementary analysis correspond to theoretical values.

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