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LEWIS ACIDS, ESPECIALLY ZINC CHLORIDE: A NEW TYPE OF CARBODIIMIDE ADDITIVE IN PEPTIDE SYNTHESIS

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In peptide synthesis using the carbodiimide method, racemisation has often been detected[1,2]. In 1966 Wünsch and Weygand[3] were able to show that the simultaneous use of dicyclohexylcarbodiimide (DCC) and two equivalents of N-hydroxysuccinimide enables peptide synthesis practically free of racemisation. Generally, based on studies of Goodman et al.[4] 1,2-dinucleophilic compounds, such as N-hydroxysuccinimide[3], 1-hydroxybenzotriazole[5], and others[6] are particularly interesting for the modified DCC method. It has been established that the oxazolone formed during DCC coupling is easily racemised by bases. For this reason, oxazolone formation and subsequent racemisation are suppressed by α-dinucleophiles and other reagents which can attack activated amino acid or peptide derivatives by biphilic mechanism[4] and they simultaneously decrease the basicity of the medium.

Contrary to the mechanism discussed we found that Lewis acids which cannot form reactive intermediates compared with hydroxylamine-derived additives show a significant influence on suppression of racemisation in DCC coupling using the sensitive gas chromatographic racemisation test of Weygand et al.[7]. The Lewis acids listed in Table 1 suppress racemisation to a higher extent than N-hydroxysuccinimide (HONSu) or 1-hydroxybenzotriazole (HOBt), respectively, which are preferentially used in the modified

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DCC procedure. On the other hand, it is worth commenting that with the exception of $ZnCl_2$ the other Lewis acids are not suitable for practical

Table 1. Studies on Racemisation in the Synthesis of Tfa-Pro-Val-Pro-OMe from Tfa-Pro-Val and Pro-OMe using the modified DCC method

Additive	Equivalent	Solvent	% L-D-L Peptide
None	-	DMF	66.8
None	-	THF	27.8
HOBt	2	DMF	9.5
HONSu	2	DMF	29.8
$ZnCl_2$	1	DMF	8.5
$ZnCl_2$	2	DMF	6.9
$ZnCl_2$	1	THF	5.2
$SnCl_4$	1	DMF	5.8
$SnCl_4$	1	THF	3.0
$TiCl_4$	1	DMF	7.2
$SbCl_3$	1	DMF	<1.0
$AlCl_3$	1	DMF	<1.0

use in peptide synthesis. Due to their properties they cannot be handled easily. Furthermore, undesired reactions with the amino component and the peptide formed as well as difficulties during the working-up procedure reduce the practical value of these additives. In order to investigate the efficiency in practical peptide synthesis we synthesized the Anderson peptide[8] Z-Gly-Phe-Gly-OEt from Z-Gly-Phe and Gly-OEt in dimethylformamide (DMF) at - 5°. Using 1 equivalent $ZnCl_2$ as additive the optically pure tripeptide was obtained in 80 % yield. Although with 1 equivalent $SbCl_3$ as additive no DL-peptide could be detected the yield was only 40 %. In comparison with the results obtained the DCC procedure without additives led to 61 % L-peptide and 13 % DL-peptide. Due to undesired reactions the use of $AlCl_3$ as additive was not successful. No crystalline product could be

isolated. For this reason, $SnCl_4$ or $TiCl_4$, respectively, have not been applied to further experiments. However, using $ZnCl_2$ as DCC additive we were able to carry out peptide synthesis with good results. In all cases studied we obtained higher yields in the simultaneous use of DCC and 1 equivalent $ZnCl_2$ as compared with DCC only. Boc-Gly-Gly-Phe-OMe from Boc-Gly and Gly-Phe-OMe, e. g., could be obtained in 70 % yield using the DCC/$ZnCl_2$ procedure whereas in synthesis without this additive the yield was only 35 %. Generally, after peptide coupling zinc chloride is removable by treatment with 0.5 N hydrochloric acid.

Beside the practical aspect of this new type of DCC additive we have been interested in the mechanism of racemisation suppression by Lewis acids. First of all, there is no doubt that Lewis acids decrease the basicity of the reaction medium. On the other hand, due to their electrophilic properties, Lewis acids may influence both the reactivity of the acyl isourea intermediate and the rate of the ring opening of the oxazolone formed. For studying the influence of Lewis acids on oxazolone racemisation we synthesized the optically active oxazolone from Z-Pro-Val. Analogously to the procedure described by Goodman and Levine[9] Z-Pro-Val was allowed to react with acetic anhydride. The reaction was followed polarimetrically. At the point of maximum negative rotation the solvent was removed and the 2-(1'-benzyloxycarbonyl-pyrrolidine-2-yl)-L-4-isopropyl-oxazolone was characterized: mp. 60 - 61.5°; $[\alpha]_D^{23} = -134.34°$ (c = 2, in dioxane); Anal. calcd. for $C_{18}H_{22}N_2O_4$: C 65.44 H 6.71 N 8.48 ; found: C 65.72 H 6.66 N 8.73. The degree of racemisation in the course of ring opening by Pro-OMe and several additives was examined. The ring opening reaction of the oxazolone was carried out in DMF at 25°. After 15 hrs the benzyloxycarbonyl group of Z-Pro-Val-Pro-OMe was removed by acidolysis with 4.5 N hydrobromic acid in glacial acetic acid. The introduction of the Tfa group was carried out by trifluoroacetic anhydride in the presence of triethylamine. The ratio of Tfa-Pro-Val-Pro-OMe to Tfa-Pro-D-Val-Pro-OMe was determined by gas chromatography. The results in Table 2 demonstrate that Lewis acids suppress the oxazolone racemisation significantly.

Table 2. Studies on Racemisation in the Synthesis of Z-Pro-Val-Pro-OMe from the Corresponding Oxazolone and Pro-OMe Using Additives in Ratio (1:1)

Additive	% L-D-L Peptide
None	40.8
Acetic acid	35.8
Pentafluorphenol	29.7
HOBt	15.2
$ZnCl_2$	7.4
$SbCl_3$	2.8

In order to explain the results obtained it seems to be necessary to investigate the rate of ring opening of the oxazolone by Pro-OMe in presence of the corresponding additives. Further studies are in progress.

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