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COPPER(II) CHLORIDE AS A NEW, EFFICIENT ADDITIVE SUPPRESSING RACEMIZATION IN PEPTIDE SYNTHESIS BY THE CARBODIIMIDE METHOD

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Summary: Copper(II) chloride was found to be an extremely efficient additive suppressing racemization in the carbodiimide mediated couplings.

The use of additives in the dicyclohexylcarbodiimide (DCC) method is an important improvement contributing to the suppression of racemization in segment couplings. Among a variety of N-hydroxy compounds proposed as such additives, 1-hydroxybenzotriazole (HOBt), for example, proved to be very effective in many peptide models and is preferably used in the practical synthesis of long peptide chains. Unfortunately, however, even the addition of this reagent cannot always guarantee couplings against racemization.[1] Thus additives preventing racemization completely are eagerly desired.

Recently we have investigated the factors which influence the degree of racemization in the model couplings of a series of benzyloxycarbonylglycylamino acids with amino acid esters, taking advantage of the good separation of diastereomers and high accuracy of analysis by reversed phase high performance liquid chromatography (RP-HPLC). During the course of this investigation we have evaluated the racemization suppressing effect of a number of additives in the carbodiimide method and have found that copper(II) chloride is an extremely efficient and promising additive as compared with the hitherto known ones. Concerning the use of such inorganic reagents as additives the racemization suppressing effect of some Lewis acids was reported several years ago.[2] However, even with the addition of zinc chloride, which was only of practical value among five Lewis acids examined, racemization still occurred to some extent in the model coupling, CF_3CO-L-Pro-L-Val + L-Pro-OMe, by the DCC method.

In order to evaluate the effectiveness of additives the model peptide coupling, Z-Gly-L-Val + L-Val-OMe, was chosen here for the following reasons. In the solvent of DMF this model system suffers extremely large degree of racemization by the DCC method and therefore must afford a stringent racemization test. And the diastereomeric pair of the resulting peptide, merely after suitable washings, can easily be separated by HPLC, by which low levels of epimer (>0.1%) can be measured accurately.

As can be seen from Table 1, practically no racemization could be detected by the simultaneous use of DCC and $CuCl_2$ (or $CuBr_2$), whereas even with the addition of HOBt a low level of racemization still occurred. In the coupling of Z-Gly-L-Val with Gly-L-Phe-OMe[3] also the addition of $CuCl_2$ virtually prevented racemization, whereas the DCC procedure without additive

Table 1. Racemization during the coupling of Z-Gly-L-Val with L-Val-OMe by the DCC method plus additive[a)]

Additive	Equiv.	D-L%	Yield (%)[b)]
None	-	43	19
HOSu[c)]	1	5.3	20
HOBt	1	0.4	45
HONB[d)]	1	5.4	20
$ZnCl_2$	1	9.1	31
$CuCl_2$	1	<0.1	27
$CuCl_2$	2	<0.1	31
$CuBr_2$	1	<0.1	24

a) Reactions were run by dissolving the carboxyl component, the amino component in the form of p-toluenesulfonate, triethylamine, and additive where pertinent (1 : 1 : 0.9 : 1 equiv.) in DMF (reactant concn, 0.018 M) and adding 1 equiv. of DCC. The duration and temperature of the reaction was 24 h at 5°C.
HPLC conditions: column, Cosmosil $5C_{18}$ (4.6 mm I.D. x 150 mm); mobile phase, 60% MeOH; flow rate, 1.0 ml/min; column temp, 30°C; detection, 254 nm.
b) Total yield of peptide diastereomers measured by HPLC with Z-Gly-L-Ala-L-Val-OMe as an internal standard, which was added to the reaction mixture before washings. c) N-Hydroxysuccinimide. d) N-Hydroxy-5-norbornene-endo-2,3-dicarboximide.

Table 2. Effect of copper(II) chloride as carbodiimide additive in the coupling of Z-Gly-L-Val with L-Val-OMe under various conditions[a)]

Carbodiimide	Reactant concn (M)	Duration and temp	D-L%	Yield (%)
DCC	0.018	5°C 24 h	<0.1	27
DCC	0.042	5°C 24 h	<0.1	51
DCC[b)]	0.018	0°C 2 h → 23°C 22 h	<0.1	59
DCC	0.042	0°C 2 h → 23°C 22 h	<0.1	74
DCC	0.083	0°C 2 h → 23°C 22 h	<0.1	81
EDC[c)]	0.018	5°C 24 h	<0.1	47

a) Unless otherwise specified reactions were run as in Table 1, using 1 equiv. of $CuCl_2$.
b) The DCC-HOBt procedure led to 2.4% D-L epimer and total yield of 96%.
c) One equiv. of EDC·HCl was used. The EDC procedure without additive led to 38% D-L epimer and total yield of 26%.

led to 32% D-L epimer. In the presence of $CuCl_2$ higher yield was obtained as compared with the case of DCC alone, though the yield itself was rather poor because of the low concentration of reactants[4)] and the low reaction temperature. The use of 2 equiv. of $CuCl_2$ resulted in only a slight increase in yield. As shown in Table 2, the yield could be raised at higher concentration of reactants and at higher temperature, while racemization remained to be prevented. The use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) with $CuCl_2$ seems to be preferable from the viewpoint of the yield. Some other transition metal compounds were also examined and found to have a significant influence on suppression of racemization in the DCC mediated couplings.

Further studies are now in progress on the practical aspect of this new type of additive and the mechanism of its racemization suppression.

References and Notes

1) D. H. Rich and J. Singh, and D. S. Kemp, "The Peptides," ed by E. Gross and J. Meienhofer, Academic Press, New York (1979), Vol. 1, Chapt. 5 and 7.
2) H.-D. Jakubke, Ch. Klessen, E. Berger, and K. Neubert, Tetrahedron Lett., 1978, 1497.
3) The amino component was used in the form of hydrobromide in the presence of 0.9 equiv. of triethylamine. The reaction was carried out in DMF at 5°C for 24 h.
4) The concentration of reactants adopted here followed the example in the literature; N. L. Benoiton and K. Kuroda, Int. J. Peptide Protein Res., 17, 197 (1981).

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