CHEMISTRY LETTERS, pp. 2125-2128, 1989.

Racemization Suppression by Copper(II) Chloride in Peptide Synthesis by the Mixed Anhydride and Related Methods

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In segment couplings by the mixed anhydride and related methods, the addition of copper(II) chloride suppressed racemization completely in the same manner as in the carbodiimide method.

We have recently demonstrated, by using a number of model systems, that in a carbodiimide-mediated segment coupling copper(II) chloride is exceptionally effective as a racemization-suppressing additive compared with other hitherto known compounds.[1,2] This remarkable racemization suppression by $CuCl_2$ can be related mechanistically to its strong ability to suppress the racemization of the 5(4H)-oxazolone which is formed from an activated carboxyl component during the coupling.[2] This suggests that $CuCl_2$ may be effective also in couplings by other methods where 5(4H)-oxazolones are primarily responsible for the observed racemization.[3] This communication reports that this is the case with couplings by the mixed anhydride and related methods.

The mixed (carbonic) anhydride method has been one of the simplest and most efficient coupling methods, but the high liability of racemization during the segment condensation has restricted its use in practical peptide synthesis.[4] Even under the "Anderson conditions" which minimize the extent of racemization by strict controlling of the coupling conditions,[5] a low level of racemization is inevitable in most cases.[4] Therefore, a more efficient procedure for the mixed anhydride method which can suppress racemization completely is still a challenge. Table 1 shows the results on the effect of $CuCl_2$ in several model segment couplings between Z-dipeptides[6] with amino acid esters by the mixed anhydride method employing isobutyloxycarbonyl chloride in DMF. In order to get a more unequivocal conclusion, more racemization-sensitive residues (in DMF) valine and isoleucine[3b] were chosen as the C-terminal residue of a carboxyl component. No detectable amount ($<0.1\%$) of the undesired epimer was observed in the presence of $CuCl_2$ (1 equiv.), regardless of the model system, although a considerable to large extent of racemization occurred in all the cases in its absence. The racemization-suppressing effect of $CuCl_2$ was outstanding compared with other compounds hitherto reported as suppressants in the mixed anhydride method.[7,8] The data (given as % D-alloisoleucine formed) obtained by employing the model

Table 1. Racemization Extent[a)] during the Coupling of Z-AA_1-AA_2-OH with H-AA_3-OMe by the Mixed Anhydride Method plus Additive[b)]

AA_1	AA_2	AA_3	Coupling time[b)]	Additive None	Additive $CuCl_2$
Gly	L-Val	L-Leu	A	7.3	<0.1
Gly	L-Ile	L-Leu	A	7.8	<0.1
Gly	L-Ile	L-Val	A	9.1	<0.1
Gly	L-Ile	L-Ile	A	9.0	<0.1
L-Ala	L-Val	L-Val	B	22	<0.1
L-Ala	L-Val	L-Leu	B	24	<0.1
L-Ala	L-Val	L-Ile	B	24	<0.1
L-Val	L-Val	L-Val	B	26	<0.1
L-Val	L-Val	L-Leu	B	23	<0.1
L-Val	L-Val	L-Ile	B	22	<0.1

a) Data are given as % D-L or L-D-L isomer formed, determined by the reversed-phase HPLC analysis of the resulting peptides (Ref. 1). b) The coupling procedure was as follows: a solution of a carboxyl component (0.25 mmol) and an additive (0.25 mmol) where pertinent in 3 ml of DMF containing NMM (0.25 mmol) was cooled to -5 °C. To this solution was added 0.25 mmol of i-BuOCOCl with stirring, followed, after 10 min, by a precooled (-5 °C) mixture of the p-toluenesulfonate of an amino component (0.25 mmol) and 2 ml of DMF containing NMM (0.25 mmol). The reaction mixture was stirred at -5 °C for 1 h and then at 20 °C for 2 h (A) or 21 h (B).

coupling Z-Gly-L-Ile-OH + H-L-Val-OMe plus additive under the same reaction conditions as in Table 1 are as follows: HOBt, 3.6; HOSu, 9.0; HONB, 9.9; PFP, 8.0; $ZnCl_2$, 8.5; ZnF_2, 9.3.

2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) has been known as a convenient substitute in the conventional mixed anhydride method;[9,4)] i.e., the compound can be used as a coupling reagent at ambient temperature. In spite of the initial claim that this reagent can minimize the possibility of racemization, it has been known that a low level of racemization do occur,[4)] the extent being dependent on the coupling system, as can be seen in the examples presented in Table 2. Here again the addition of $CuCl_2$ suppressed racemization completely as in the conventional mixed anhydride method, even at ambient temperature where EEDQ was used.

Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) has recently been proposed as a powerful coupling reagent which is applicable even to the couplings between N-methylamino acid residues.[10)] The reactions are thought to proceed via mixed carboxylic-phosphinic anhydrides. Racemization during the segment coupling by this reagent has also been examined by using the model coupling Z-Gly-L-Phe-OH

+ H-L-Val-OMe: HOBt was found to be most effective among the additives tested, but it could not eliminate racemization completely in DMF.[10b)] This was confirmed further by using a more sensitive test system Z-Gly-L-Val-OH + H-L-Val-OMe plus additive in DMF. The percentages of the undesired D-L epimer formed are as

Table 2. Racemization Extent[a)] during the Coupling of Z-AA_1-AA_2-OH with H-AA_3-OMe by the EEDQ Method plus Additive[b)]

AA_1	AA_2	AA_3	Additive None	 $CuCl_2$
Gly	L-Val	L-Val	1.2	<0.1
Gly	L-Ile	L-Val	2.2	<0.1
L-Ala	L-Val	L-Ala	1.4	<0.1
L-Ala	L-Val	L-Val	2.4	<0.1
L-Ala	L-Val	L-Leu	2.5	<0.1
L-Val	L-Val	L-Ala	3.2	<0.1

a) Data given as % D-L or L-D-L isomer formed. b) Reactions were run by dissolving a carboxyl component, the p-toluenesulfonate of an amino component, an additive where pertinent, and triethylamine (1 : 1 : 1 : 1 equiv.) in DMF (reactant concentration, 0.05 M), and adding 1 equiv. of EEDQ. The duration and temperature of the reaction was 24 h at 20 °C.

Table 3. Racemization Extent[a)] during the Coupling of Z-AA_1-AA_2-OH with H-AA_3-OMe by the BOP-Cl Method plus Additive[b)]

AA_1	AA_2	AA_3	Additive None	 HOBt	 $CuCl_2$
Gly	L-Phe	L-Val	14	2.8	<0.1
Gly	L-Val	L-Val	33	3.7	<0.1
Gly	L-Ile	L-Val	40	4.5	<0.1
L-Ala	L-Val	L-Ala	26	3.8	<0.1
L-Ala	L-Val	L-Val	31	4.0	<0.1

a) Data given as % D-L or L-D-L isomer formed. b) Reactions were run by dissolving a carboxyl component, the p-toluenesulfonate of an amino component, an additive where pertinent, and NMM (1 : 1 : 1 : 3.2 equiv.) in DMF (reactant concentration, 0.05 M), and adding 1.2 equiv. of BOP-Cl at 0 °C (Cf. Ref. 10b). The reaction mixture was stirred at 0 °C for 1 h and then at 20 °C for 24 h.

follows: HOBt, 3.7; HOSu, 28; HONB, 27; PFP, 36; imidazole, 28; $ZnCl_2$, 30. By contrast, the addition of $CuCl_2$ virtually prevented racemization (<0.1% D-L epimer). This remarkable racemization-suppressing effect of $CuCl_2$ in the BOP-Cl method was confirmed further by employing some other model couplings (Table 3). In every coupling system, a low level of racemization still occurred even with the addition of HOBt, while $CuCl_2$ suppressed racemization completely.

The present results are consistent with the above-mentioned view on the mechanism of racemization suppression by $CuCl_2$, and its effect in other coupling methods is now under investigation.

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6) Abbreviations: Z, benzyloxycarbonyl; DMF, N,N-dimethylformamide; NMM, N-methylmorpholine; HOBt, 1-hydroxybenzotriazole; HOSu, N-hydroxysuccinimide; HONB, N-hydroxy-5-norbornene-endo-2,3-dicarboximide; PFP, pentafluorophenol.
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(Received September 1, 1989)