Racemization-free and Efficient Peptide Synthesis by the Carbodiimide Method using 1-Hydroxybenzotriazole and Copper(II) Chloride simultaneously as Additives

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In carbodiimide mediated segment couplings, the simultaneous use of 1-hydroxybenzotriazole and copper(II) chloride as additives was found to give racemization-free peptides in high yields.

The prevention of racemization during segment couplings is one of the most serious problems to be solved urgently in peptide synthesis.[1] The introduction of racemization-suppressing additives has been one of the most important improvements in the carbodiimide method. Among a variety of compounds proposed as such additives, 1-hydroxybenzotriazole (HOBt), for example, has most frequently been used in practical syntheses. Unfortunately, however, even with this additive, couplings are not always free from racemization.[2] We recently reported the remarkable ability of copper(II) chloride to suppress racemization in the carbodiimide method by employing the model coupling Z-Gly-L-Val-OH (Z = benzyloxycarbonyl) + H-L-Val-OMe.[3] However, the coupling yields were not necessarily satisfactory. In this communication we propose a new, promising procedure using HOBt and $CuCl_2$ simultaneously as additives, which can afford racemization-free peptides in high yields.

The effectiveness of $CuCl_2$ as a racemization suppressant was further confirmed by employing the 24 other couplings Z-Gly-L-AA_1-OH + H-L-AA_2-OMe, where AA_1 or AA_2 denotes any amino acid residue among Ala, Val, Leu, Ile, and Phe. When $CuCl_2$ (1 equiv.) was used as a 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) additive in dimethyl formamide (DMF),† reversed-phase h.p.l.c. analysis[3] of the resulting peptides showed no detectable amount of the D–L epimer (<0.1%) in all except two cases (0.3% each for -Phe + Leu and -Ile + Ile).‡ Furthermore, even in the EDC mediated condensation in DMF of Z-L-Pro-L-Val-OH with H-L-Pro-OMe,[4] no racemization was observed in the presence of $CuCl_2$ (1 equiv.), while the L–D–L epimer increased to 15% with HOBt as an additive.

Next, the model coupling Z-Gly-L-Val-OH + H-L-Val-OMe was reinvestigated from the viewpoint of both racemization suppression and coupling efficiency (Table 1). No racemization was observed in the EDC mediated couplings with the addition of $CuCl_2$ (≥0.5 equiv.), while the coupling yields were unsatisfactory. In contrast, the use of HOBt gave an extremely high yield, but a low level of racemization was inevitable. These results prompted us, as a new method, to use HOBt and $CuCl_2$ simultaneously as additives, in expectation of eliminating each defect complementarily. The simultaneous use of HOBt and $CuCl_2$ (1 equiv. each), for example, gave the desired peptide in a fairly good yield, while racemization remained undetectable. In the presence of HOBt, reducing the amount of $CuCl_2$ gave a higher yield. Besides the improvement of coupling efficiency, the present procedure offered another advantage to racemization suppression. Thus, even in the above mentioned couplings, Z-Gly-L-Phe-OH + H-L-Leu-OMe and Z-Gly-L-Ile-OH + H-L-Ile-OMe, the simultaneous addition of HOBt and $CuCl_2$ (1 equiv. each) prevented racemization (<0.1% D–L epimer).

In order to assess the effectiveness of this new way of using the two carbodiimide additives in the synthesis of biologically active peptides, the preparation of a protected Leu-enkephalin was carried out by segment condensation Boc-L-Tyr(Bzl)-Gly-Gly-L-Phe-OH, (Boc = t-butoxycarbonyl; Bzl = benzyl) and H-L-Leu-OMe.[5] As shown in Table 2, a low level of the undesired epimer was detected when $CuCl_2$ alone was used as an EDC additive, similarly to the above mentioned model coupling Z-Gly-L-Phe-OH + H-L-Leu-OMe; the extent of racemization, however, was smaller than that with HOBt as an additive. As expected, in the couplings using HOBt and $CuCl_2$ simultaneously, no racemization was detected and the yields were high enough.

Table 1. Coupling of Z-Gly-L-Val-OH with H-L-Val-OMe by the EDC method plus additives in DMF.[a]

HOBt equiv.	$CuCl_2$ equiv.	% D–L	% Yield[b]
0	0	38	22
0	0.5	<0.1	26
0	1	<0.1	40
1	0	1.3	96
1	0.25	<0.1	79
1	0.5	<0.1	74
1	1	<0.1	73
2	0.1	<0.1	88
2	0.25	<0.1	83
2	0.5	<0.1	75
2	1	<0.1	70

[a] The reactions were run as before (ref. 3), for 24 h at 5 °C. After the addition of an internal standard and subsequent washes, the product was analysed by reversed-phase h.p.l.c. [b] Total yield of peptide epimers. Yields reported previously (ref. 3) should be multiplied by a factor of 0.85 for correction.

Table 2. Coupling of Boc-L-Tyr(Bzl)-Gly-Gly-L-Phe-OH with H-L-Leu-OMe by the EDC method plus additives in DMF.[a]

HOBt equiv.	$CuCl_2$ equiv.	% L–D–L	% Yield[b]
0	0	20	37
0	1	0.5	24
1	0	0.9	94
1	0.5	<0.1	90
1	1	<0.1	84
2	0.25	<0.1	88
2	0.5	<0.1	91
2	1	<0.1	88

[a] Reaction for 24 h at 5 °C. Product analysed by reversed-phase h.p.l.c. [b] Total yield of peptide epimers.

† In the coupling Z-Gly-L-Val-OH + H-L-Val-OMe, EDC·HCl gave a better yield than dicyclohexylcarbodiimide (DCC) when $CuCl_2$ was used as an additive (see ref. 3) (24 h; 5 °C).

‡ 0.8% D–L epimer for -Phe + Leu and 1.6% for -Ile + -Ile in the presence of HOBt as an additive.

In reference to the mechanism of racemization suppression by $CuCl_2$, it was found that the optical rotation[6] of the oxazol-5(4*H*)-one from Z-Gly-L-Val-OH was maintained constant over ≥3 h in the presence of $CuCl_2$ (1 equiv.) in DMF. Furthermore, even when the addition of H-L-Val-OMe was delayed after Z-Gly-L-Val-OH had been treated with EDC·HCl for 2 h in DMF, the D–L epimer was not formed if $CuCl_2$ (1 equiv.) was present during the activation stage.§ These results indicate that $CuCl_2$ has a strong ability to suppress the racemization of the oxazol-5(4*H*)-one which may be formed from an activated carboxy component during the coupling.[7] Therefore, the elimination of racemization and improvement of coupling efficiency produced by the simultaneous addition of HOBt and $CuCl_2$ in the carbodiimide method must be attributable to a reduced tendency for an activated carboxy component such as an *O*-acylisourea intermediate to form an oxazol-5(4*H*)-one by the action of HOBt,[1] and to the prevention of racemization by $CuCl_2$ of a small, if any, amount of the oxazol-5(4*H*)-one formed. Moreover, HOBt can suppress side reactions such as the rearrangement of an *O*-acylisourea intermediate to an *N*-acylurea derivative, contributing to the increase of coupling yield.

§ Other results: 42% D–L epimer without $CuCl_2$, 12% with HOBt.

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