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Efficient Coupling of α,α-Dimethyl Amino Acid using a New Chloro Imidazolidium Reagent, CIP

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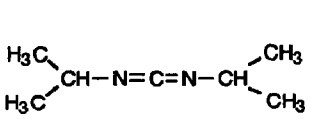
Abstract: CIP (2-chloro-1,3-dimethylimidazolidium hexafluorophosphate) was an efficient coupling agent for Nα-protected *a*-aminoisobutyric acid (Aib) in the presence of an additive. The reactivity was enhanced markedly by a catalytic amount of additive in the order of HOAt~HODhbt>DMAP>HOBt. These couplings occurred without detectable racemization.

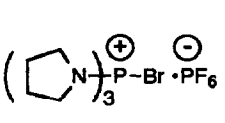
Coupling of α,α-dimethyl amino acid (Aib, α-aminoisobutyric acid) is one of the most difficult reactions both in solution- and solid-phase peptide syntheses[1]. The usual activation as symmetrical-anhydride, mixed-anhydride, or active ester has been reported to give a substantially low coupling yield despite a long reaction time[2]. For this reason, few methods of chemical synthesis of Aib-containing peptides have been reported, although Aib-containing antibiotics (peptaibols) have interesting biological and conformational properties[3]. To overcome this difficulty, a pre-activated form of Nα-protected Aib (fluoride[4] or N-carboxyanhydride[2]) and a coupling reagent (PyBroP[1]), have been developed and applied for the model-peptide syntheses.

Recently, we reported a procedure for the esterification of Fmoc amino acid derivative to 4-alkoxybenzylalcohol resin using a new esterification reagent, CIP (2-chloro-1,3-dimethylimidazolidium hexafluorophosphate)[5]. The reaction proceeded rapidly with the same or lower racemization level than the anchoring reaction using the conventional esterification reagents. Considering that the coupling of free acid using a coupling reagent would be more convenient and generally applicable than the pre-activated form of α,α-dialkylamino acids, we examined the use of CIP as a coupling reagent for Aib-containing peptides. Herein, we report that CIP, in the presence of additive, was an efficient coupling agent for Nα-protected Aib.

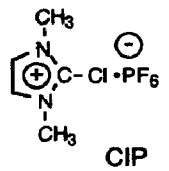
To evaluate the coupling efficiency, three types of model dipeptides were synthesized using CIP as



DIPCDI



PyBroP



CIP

Table 1. Yields (%) of dipeptides obtained by the different coupling methods

	R=Boc			R=Z			R=Fmoc		
	PyBroP	CIP	CIP/ HOAt	PyBroP	CIP	CIP/ HOAt	PyBroP	CIP	CIP/ HOAt
R-Aib-Val-OMe*	37	43	82	60	59	92	57	59	93
R-Aib-Aib-OMe*	4	6	80	17	11	82	12	10	90
R-Val-Aib-OMe*	41	44	80	60	60	85	50	51	87

* All couplings were conducted at 25°C for 60 min. Each synthesized dipeptide was isolated and purified by silica-gell column chromatography to give a crystal.

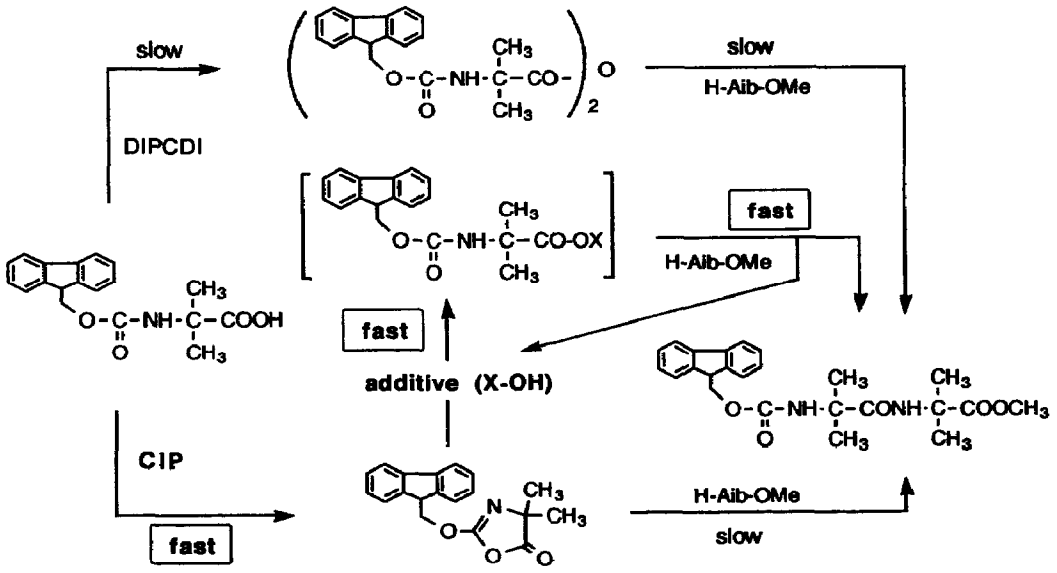
a coupling reagent; R-Aib-Val-OMe, R-Val-Aib-OMe, and R-Aib-Aib-OMe (R=Boc, Z, or Fmoc) (Table 1). Coupling proceeded at 25°C for 60 min without additive. For comparison, the same model peptide was prepared under the same conditions using PyBroP as a coupling reagent. Each desired dipeptide was isolated from the reaction mixture as a crystal. For the Aib-Val and Val-Aib sequences, the yields were moderate when Z or Fmoc was employed as the N^{α}-protecting group, whereas N^{α}-Boc amino acids gave lower yields than those obtained for Z- and Fmoc-dipeptides. These low yields can be explained by the formation of the corresponding N-carboxyanhydride (NCA) of Boc-amino acid as described by Frérot et al[6]. This side-reaction is not observed with the Z- and Fmoc-protecting groups. In the preparation of Aib-Aib sequence, extremely low yields were obtained for all three N^{α}-protecting groups. To improve the yields of CIP-coupling, we then examined the effects of several additives, i.e, DMAP, HOBt, HODhbt[7], and HOAt[8]. All additives examined markedly enhanced the reactivity in catalytic amount. Table 1 shows the yields obtained by addition of HOAt in coupling of CIP[9]. The dipeptides including N^{α}-Boc peptides were obtained in excellent isolation yields.

Racemization during the coupling of R-Val-OH with H-Aib-OMe was examined by GC analysis after hydrolysis of the synthesized dipeptide. The hydrolysate was derivatized as (pentafluoropropionyl)amino acid n-butyl ester, which was analyzed on a Chirasil Val-L capillary column. The D-Val contents of the dipeptides prepared by the CIP/HOAt procedure were less than 0.5 %; this shows that the coupling can be conducted without detectable racemization.

To estimate the reaction mechanism of the CIP-coupling, two expected intermediates, Fmoc-Aib anhydride and 2-[(9-fluorenylmethyl)oxy]-4-dimethyl-5-oxazolone, were prepared by the treatment of Fmoc-Aib-OH with DIPCDI (25°C, 2 h). Both compounds were isolated and purified by silica-gel column chromatography and characterized by ^{1}H-NMR (270 MHz) and ^{13}C-NMR (67.9 MHz)[10]. From the comparison of ^{1}H-NMR chemical shifts of the two compounds, we could identify the upfield shift of methyleneoxy protons of the oxazolone derived from Fmoc-Aib-OH as described earlier for the oxazolone derived from Fmoc-Val-OH[11]. This upfield shift makes it possible to estimate each amount of Fmoc-Aib-OH, its anhydride, and the oxazolone in the reaction mixture using ^{1}H-NMR.

Thus, Fmoc-Aib-OH in $CDCl_3$ was treated with CIP and the mixture was examined periodically by

Scheme 1



^{1}H-NMR. For comparison, activation with DIPCDI and PyBroP was also examined. In the activation with DIPCDI, slow formation of the anhydride (60 % after 19 h) and no significant formation of the oxazolone were detected. By contrast, immediate transformation to the oxazolone (ca 90 % after 15 min) was detected by CIP activation; 60 % transformation to the oxazolone was detected when PyBroP was used for activation. The kinds of intermediates and their formation rates were clearly different between these three coupling reagents. In the preparation of Fmoc-Aib-Val-OMe and Fmoc-Aib-Aib-OMe using CIP in $CDCl_3$, we could detect gradual disappearance of the oxazolone-methyleneoxy peaks on ^{1}H-NMR. The rates of the peak disappearance were in agreement with the isolation yields shown in Table 1.

By the same monitoring method using ^{1}H-NMR, the catalytic enhancement effects of the additives for the CIP-coupling were also examined; the order of enhancement was HOAt~HODhbt>DMAP>HOBt. In the preparation of Fmoc-Aib-Aib-OMe, the coupling rate obtained by the addition of 0.5 equiv. of HOBt was ca 9 times faster and that obtained by the addition of 0.25 equiv. of HOAt was ca 33 times faster than the rate without additives. The oxazolone derived peaks disappeared almost completely within 20 min in CIP coupling in the presence of HOAt, HODhbt, or DMAP. In the CIP/HOBt coupling, fast formation of another intermediate and its gradual disappearance was detected on ^{1}H-NMR spectrum. The intermediate would be the oxybenzotriazole ester of Fmoc-Aib.

These findings suggest that the CIP-coupling proceeds according to the pathway shown in Scheme 1. The oxazolone of Fmoc-Aib formed through CIP activation is transformed to a highly active intermediate by a catalytic amount of the additive, then the active intermediate ester quickly reacts with H-Aib-OMe to give Fmoc-Aib-Aib-OMe. The direct reaction of the oxazolone with an amine component is slow to moderate depending on the reactivity of the amine.

In conclusion, the CIP in the presence of additive is a suitable coupling agent for Aib including Nα-Boc protected Aib. In the coupling using CIP and an additive, the first intermediate, 2-[(9-fluorenylmethyl)oxy]-4-dimethyl-5-oxazolone, is transformed to the highly active ester by the catalytic additive to give a desired peptide without detectable racemization.

Abbreviations

Boc=t-butyloxycarbonyl, Fmoc=fluoren-9-ylmethyloxycarbonyl, Z=benzyloxycarbonyl, DIPCDI=diisopropylcarbodiimide, DIEA=diisopropylethylamine, PyBroP=bromotripyrrolidino phosphonium hexafluorophosphate, HOBt=N-hydroxybenzotriazole, DMAP=4-dimethylaminopyridine, HOAt=1-hydroxy-7-azabenzotriazole, HODhbt=3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine.

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8. Carpino, L.A. *J. Am. Chem. Soc.*, **1993**, 115, 4397-4398. HOAt used in this report is a kind gift from Dr. F. Albericio at Millipore Co. (Bedford, Massachusetts).
9. Typical experiment: To the suspension of Fmoc-Aib-OH (0.5 mmol) and HOAt (0.25 equiv.) in CH_2Cl_2 (4 ml) is added DIEA (4 equiv.) at an ice-bath temperature. Then, the addition of CIP (1 equiv.) to the mixture is followed by that of 1.1 equiv. of HCl•H-Aib-OMe. After stirring for 60 min at 25°C, classical work-up followed by chromatography on silica-gel gives the crystalline dipeptide.
10. Anhydride: ^{1}H-NMR($CDCl_3$); δ 7.76 (d, J=7.59Hz, 2H), 7.60 (d, J=7.26Hz, 2H), 7.26-7.43 (m, 4H), 4.39 (d, J=6.77 Hz, 2H), 4.22 (t, J=6.77Hz, 1H), 1.54 (s, 6H). ^{13}C-NMR ($CDCl_3$); δ 175.1, 154.9, 144.0, 141.3, 127.7, 127.1, 125.0, 120.0, 66.5, 56.5, 47.2, 25.1.
    Oxazolone: ^{1}H-NMR ($CDCl_3$); δ 7.78 (d, J=7.59Hz, 2H), 7.64 (d, J=7.59Hz, 2H), 7.26~7.46 (m, 4H), 4.56 (d, J=7.26Hz, 2H), 4.38 (t, J=7.26Hz, 1H), 1.43 (s, 6H). ^{13}C-NMR ($CDCl_3$); δ 178.8, 157.0, 142.8, 141.4, 128.1, 127.2, 125.3, 120.2, 71.9, 66.8, 46.4, 25.3.
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