An Efficient Synthetic Method of N-Protected Dipeptide Acids Using Amino Acid Calcium Carboxylates in an Organic Solvent

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Abstract: The syntheses of N-protected dipeptide acids using alkaline earth metal (Mg, Ca, and Ba) carboxylates of an amino acid in organic solvents were investigated. It was found that amino acid calcium carboxylates are the most effective among the carboxylates of the amino acids tested for coupling with active esters of Boc-Ala-OH in organic solvents. The coupling of Boc-Ala-ONp or Boc-Ala-ONSu with amino acid calcium carboxylates in DMF gave the desired N-protected dipeptide acids in high yields (92–100%).

Key words: N-protected peptide acid, amino acid calcium carboxylate, organic solvent, active ester method, synthesis

N-Protected peptide acids are intermediates available as a carboxyl component for fragment condensation in peptide synthesis. In the synthesis of N-protected peptide acids, the carboxyl group of an amino component is usually protected by an ester which is removed after the coupling with the carboxyl component.[1] In general, when peptide esters are treated with deblocking reagents, incomplete removal of the esters or unexpected side reactions can occur. In contrast, protection of the carboxyl group by a metal ion can easily be achieved by dissolving amino acids in an aqueous solution of metal hydroxides; the metal carboxylates are converted into the free carboxyl groups by acidification of the reaction mixture after the coupling. Therefore, not only does the protection of a carboxyl group by a metal ion shorten the time for introduction and removal of the protecting group, but also side reactions caused by acid- or base-catalyzed deprotection, and so on, can be prevented. Coupling using amino acid–alkaline metal salts as an amino component has been carried out in an aqueous organic solvent, as these salts are insoluble in organic solvents.[1] However, the desired peptides are not obtained in satisfactory yield. As means to increase the solubility of amino acid carboxylates in an organic solvent, methods using tertiary amines[1] and phase-transfer reagents[2,3] have been reported. However, the disadvantage of these methods is that the amino acid carboxylates cannot be prepared in advance, and also phase-transfer reagents are relatively expensive.

Since alkaline earth metals form a bivalent ion, one of the metal ions combines with two molecules of an amino acid or a peptide having a free carboxyl group as shown in Scheme 1. Consequently, the amino acid or the peptide carboxylates protected by the alkaline earth metal ions could be expected to possess a higher hydrophobicity compared to the corresponding carboxylates protected by alkaline metal ions. Therefore, we anticipated that the use of alkaline earth metal carboxylates of amino acid will enable the condensation with the carboxyl components in organic solvents and that also dipeptide salts, (Boc-Ala-AA)$_2$M, generated by the coupling would be liable to dissolve in organic solvents. In this paper, we report on the effectiveness of amino acid calcium carboxylates for the synthesis of N-protected dipeptide acids.

The general synthetic procedure of N-protected dipeptide acids using amino acid alkaline earth metal salts as an amino component was carried out according to the method shown in Scheme 1. An N-protected amino acid active ester, Boc-Ala-X,[4] was dissolved in an organic solvent. To this solution was added an amino acid alkaline earth metal salt, (H-AA-O)$_2$M.[5] After the coupling at 25 °C for a certain period of time, the yield of the obtained N-protected dipeptide acid was determined by isolation and HPLC using an internal standard method.[6]

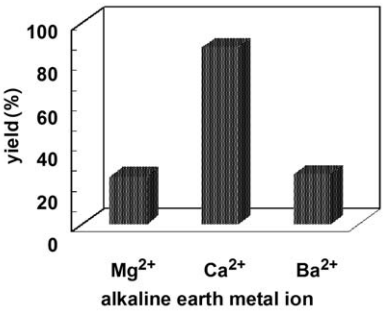

Figure 1 Effect of alkaline earth metal ions, M^{2+}, in the synthesis of Boc-Ala-Leu-OH by the coupling of Boc-Ala-ONp with (H-Leu-O)$_2$M. Yields were determined by HPLC. *Reagents and conditions*: Boc-Ala-ONp (0.30 mmol), (H-Leu-O)$_2$M (0.165 mmol), DMF (4.0 mL), 25 °C, and 1.0 h.

The reactivity of the amino acid alkaline earth metal salts, (H-AA-O)$_2$M (M: Mg, Ca, or Ba), which were prepared from the corresponding metal hydroxides, Mg(OH)$_2$, Ca(OH)$_2$, or Ba(OH)$_2$, was examined for the coupling between (H-Leu-O)$_2$M and the Boc-Ala-*p*-nitrophenyl ester, Boc-Ala-ONp, in dimethylformamide (DMF) at 25 °C for one hour. These results are shown in Figure 1. The cou-

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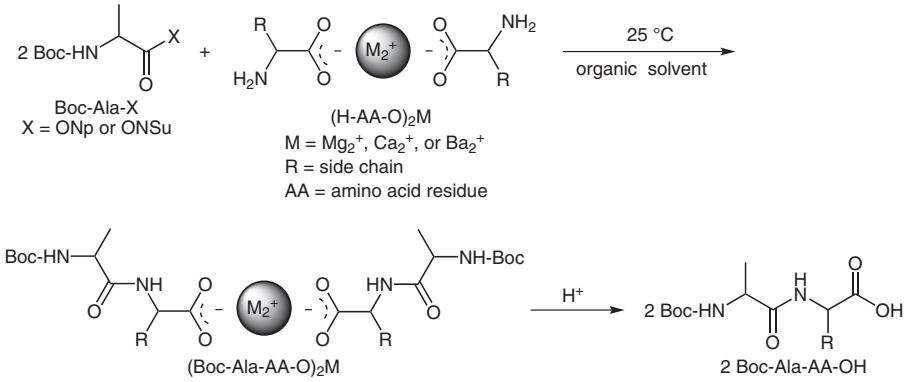

Scheme 1 Synthetic scheme of Boc-Ala-AA-OH by the coupling of Boc-amino acid active esters with amino acid alkaline earth metal salts in an organic solvent. *Reagents and conditions*: Boc-Ala-X (1.0 equiv), (H-AA-O)$_2$M (0.55 equiv), organic solvent (4.0–20 mL), 25 °C, and a certain period of time.

pling of Boc-Ala-ONp with (H-Leu-O)$_2$Ca gave the desired N-protected dipeptide acid, Boc-Ala-Leu-OH, in high yield (88%), but the couplings of Boc-Ala-ONp with (H-Leu-O)$_2$Mg or (H-Leu-O)$_2$Ba resulted in the N-protected dipeptide acid in low yields (24% and 25%, respectively). These results indicated that the amino acid calcium carboxylate is the most effective among the salts tested.

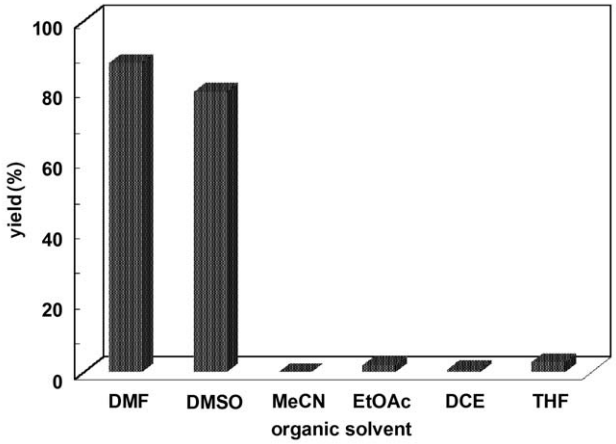

Figure 2 Effect of the organic solvents in the synthesis of Boc-Ala-Leu-OH by the coupling of Boc-Ala-ONp with (H-Leu-O)$_2$Ca. Yields were determined by HPLC. *Reagents and conditions*: Boc-Ala-ONp (0.30 mmol), (H-Leu-O)$_2$Ca (0.165 mmol), solvent (4.0 mL), 25 °C, and 1.0 h.

The effect of organic solvents in the syntheses of the N-protected dipeptide acids was evaluated by the coupling of Boc-Ala-ONp with (H-Leu-O)$_2$Ca at 25 °C for one hour. Figure 2 shows the yields from the coupling in various organic solvents. In highly polar aprotic solvents such as DMF and dimethylsulfoxide (DMSO), Boc-Ala-Leu-OH was formed in high yields (88% and 80%, respectively). The organic solvents such as acetonitrile (MeCN), ethyl acetate (EtOAc), 1,2-dichloroethane (DCE), and tetrahydrofuran (THF) provided the desired N-protected dipeptide acid in very low yields (<1%, 2%, 1%, and 3%, respectively).

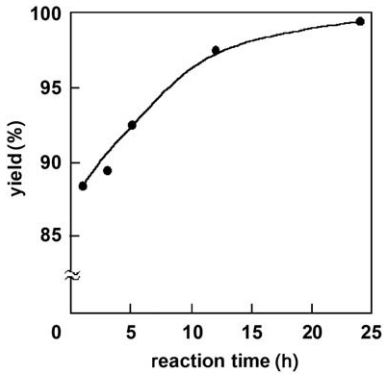

Figure 3 Effect of the reaction time in the synthesis of Boc-Ala-Leu-OH by the coupling of Boc-Ala-ONp with (H-Leu-O)$_2$Ca. Yields were determined by HPLC. *Reagents and conditions*: Boc-Ala-ONp (0.30 mmol), (H-Leu-O)$_2$Ca (0.165 mmol), DMF (4.0 mL), and 25 °C.

The effect of the reaction time for the formation of the N-protected dipeptide acids was examined by the coupling between Boc-Ala-ONp and (H-Leu-O)$_2$Ca in DMF at 25 °C. Figure 3 shows the yields for each reaction time. Although a yield of over 90% was obtained by the reaction for five hours, subsequent experiments were performed with a reaction time of 24 hours because having as little starting material as possible remaining in the reaction mixture can be expected to facilitate the isolation of the desired N-protected dipeptide acids.

Various amino acid calcium carboxylates and amino acid derivative calcium carboxylates as an amino component were coupled with Boc-Ala-ONp in DMF at 25 °C for 24 hours. The yields by HPLC are shown in Table 1. The yields from the couplings of the calcium salts of Gly and Ala having a small side chain were 100% and 92%, respectively, and the yields from the couplings of the calcium salts of Leu and Ile having a bulky side chain were 99% and 100%, respectively. The use of the calcium salt of Phe, which has a benzene ring at the side chain, also provided the desired peptide in a 98% yield. The yield from the coupling of the calcium salt of Pro, which is an α-imino acid with a cyclic structure, was 98%. In the case

Table 1 Synthesis of Boc-Ala-AA-OH by the Coupling of Boc-Ala-ONp with $(H\text{-}AA\text{-}O)_2Ca$

AA	Yield (%)		$[\alpha]_D^{22}$ (°)[c]
	HPLC[a]	Isolation[b]	
Gly	100	94	–23.9[d]
Ala	92	92	–36.9
Leu	99	97	–41.3[e]
Ile	100	99	–25.1
Phe	98	96	–1.07
Pro	98	98	–92.5[f]
Ser(Bzl)	96	93	+8.50
Lys(Z)	100	98	–10.1

[a] Yields were determined by HPLC. *Reagents and conditions*: Boc-Ala-ONp (0.30 mmol), $(H\text{-}AA\text{-}O)_2Ca$ (0.165 mmol), DMF (4.0 mL), 25 °C, 24 h.
[b] Yields were determined by isolation. *Reagents and conditions*: Boc-Ala-ONp (1.50 mmol), $(H\text{-}AA\text{-}O)_2Ca$ (0.825 mmol), DMF (5.0–20 mL), 25 °C, and 24 h.
[c] Solvent: MeOH, $c = 1.00$.
[d] Ref. 9.
[e] Ref. 10.
[f] $[\alpha]_D^{28}$ –93.9° (MeOH, $c = 1.00$). Ref. 9.

of $[H\text{-}Ser(Bzl)\text{-}O]_2Ca$ and $[H\text{-}Lys(Z)\text{-}O]_2Ca$, which has a hydroxy group and an amino group at the side chain, protected by a benzyl (Bzl) and benzyloxycarbonyl (Z), respectively, the yields were 96% and 100%, respectively. In all the examples tested, the application of amino acid calcium carboxylates gave the N-protected dipeptide acids in almost quantitative yield. Also, the isolated yields in these couplings and the values of the specific rotation for each compound are shown in Table 1. It was possible to isolate the desired N-protected dipeptide acids in high yields (92–99%).[7] Such excellent yields will suggest that $(H\text{-}AA\text{-}O)_2Ca$ in the present experiment does not form a chelate structure.[8] In these couplings, the reaction solutions became nearly clear with the progress of the reaction, namely, this result indicated that the resulting dipeptide calcium salts, $(Boc\text{-}Ala\text{-}AA\text{-}O)_2Ca$, shown in Scheme 1 are highly soluble in DMF. Such solubility of $(Boc\text{-}Ala\text{-}AA\text{-}O)_2Ca$ in DMF may be the reason for the excellent yields in those couplings. The results of the specific rotation confirmed that no racemization has taken place in the couplings using $(H\text{-}AA\text{-}O)_2Ca$.[9,10]

The condensation of various amino acid calcium carboxylates with the Boc-Ala-*N*-hydroxysuccinimide ester, Boc-Ala-ONSu, as a carboxyl component, was performed under similar conditions to the method employing ONp. As can be seen in Table 2, each coupling also gave the desired N-protected dipeptide acids in high yields (94–100%). These results showed that the use of the calcium carboxylates of amino acid in the synthesis of N-protected dipeptide acids is efficiently applicable to both the ONp and the ONSu methods.

Table 2 Synthesis of Boc-Ala-AA-OH by the Coupling of Boc-Ala-ONSu with $(H\text{-}AA\text{-}O)_2Ca$[a]

AA	Yield (%)
Gly	100
Ala	96
Leu	97
Ile	100
Phe	94

[a] Yields were determined by HPLC. *Reagents and conditions*: Boc-Ala-ONSu (0.30 mmol), $(H\text{-}AA\text{-}O)_2Ca$ (0.165 mmol), DMF (4.0 mL), 25 °C, and 24 h.

In conclusion, it was found that a variety of amino acid calcium carboxylates and/or amino acid derivative calcium carboxylates are extremely efficient as amino components in the synthesis of the N-protected dipeptide acids using the active esters, Boc-Ala-ONp and Boc-Ala-ONSu, in DMF, and that the present method gives the desired N-protected dipeptide acids in almost quantitative yield by the use of a small excess of those calcium salts. Also, it is advantageous that those amino acid calcium salts are easy to prepare, and preserve very well. Therefore, this synthetic method may prove a very useful tool for the synthesis of a small amount of stable isotope-labeled N-protected dipeptide acids and dipeptides, and also for the synthesis of those dipeptides on an industrial scale and at low cost.

References and Notes

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(4) The amino acids used were of L-configuration. The abbreviations used are those recommended by the IUPAC-IUB Joint Commission on Biochemical Nomenclature and Symbolism for Amino Acids and Peptides. See:*Eur. J. Biochem.* **1984**, *138*, 9.
(5) **Typical Procedure for the Preparation of Amino Acid Calcium Carboxylates; $(H\text{-}Leu\text{-}O)_2Ca$**: To a solution of H-Leu-OH (2.623 g, 20.0 mmol) dissolved in distilled H_2O (100 mL) was added a solution of $Ca(OH)_2$ (0.815 g, 11.0 mmol) dissolved in distilled H_2O (50 mL). After the mixture was stirred for 30 min at r.t., the H_2O was removed by evaporation. The obtained solid was dried over SiO_2 for 1 d in vacuo. The solid was ground into a powder using a mortar and pestle. The powder, $(H\text{-}Leu\text{-}O)_2Ca$, was washed onto a filter funnel with THF and Et_2O, and then dried over SiO_2 in vacuo. Yield: 2.943 g (98%).
(6) **Typical Procedure for the Determination of HPLC Yield of N-Protected Dipeptide Acids; Boc-Ala-Leu-OH**: To a solution of Boc-Ala-ONp (0.0931 g, 0.300 mmol) dissolved in DMF (4.0 mL) was added $(H\text{-}Leu\text{-}O)_2Ca$ (0.0496 g, 0.165 mmol). The mixture was stirred at 25 °C for 24 h. To the mixture were then added MeCN (10 mL), H_2O (10 mL), and Boc-Val-OH (0.300 g, 1.38 mmol) as the internal standard

reagent. The solution was applied to an HPLC column under the conditions described below. From the HPLC result, the yield of the product, Boc-Ala-Leu-OH, was determined using a calibration curve with a straight line plotted against three different concentration ratios of Boc-Ala-Leu-OH and Boc-Val-OH. Yield: 99%. t_R (Boc-Ala-Leu-OH) = 21.9 min, and t_R (Boc-Val-OH) = 16.4 min. HPLC conditions: column, Waters μ-Bondasphere (3.9 × 150 mm, C18, 5 μm, and 300 Å); solvent: 14–41% MeCN–H_2O–0.1% TFA; linear gradient, 60 min; flow rate: 1.0 mL/min; and detection, λ = 210 nm.

(7) **Typical Procedure for the Determination of Isolation Yield of N-Protected Dipeptide Acids; Boc-Ala-Ser(Bzl)-OH**: To a solution of Boc-Ala-ONp (0.465g, 1.50 mmol) dissolved in DMF (5.0 mL) was added [H-Ser(Bzl)-O]$_2$Ca (0.354g, 0.825 mmol). After the mixture was stirred at 25 °C for 24 h, the solution was condensed in vacuo. The residue was then dissolved in EtOAc (ca. 100 mL). The organic layer was washed with 5% aq citric acid (1 × 20 mL, 3 × 10 mL) and H_2O (5 × 10 mL), and then dried over anhyd Na_2SO_4. After the removal of the solid, the filtrate was condensed in vacuo. The residue was recrystallized from EtOAc–hexane. Yield: 0.511 g (93%); mp 155.4–156.0 °C. MS (FAB): m/z = 337 $[M + H]^+$ and 359 $[M + Na]^+$. t_R = 23.3 min. HPLC conditions: column, Waters μ-Bondasphere (3.9 × 150 mm, C18, 5 μm, and 300 Å); solvent: 5.0–90.5% MeCN–H_2O–0.1% TFA; linear gradient, 60 min; flow rate, 1.0 mL/min; and detection, λ = 210 nm.

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(9) For the values of specific rotation, see: (a) Boc-Ala-Gly-OH·H_2O: $[\alpha]_D^{27}$ –23.2° (MeOH, *c* = 2.0): Wolman, Y.; Schwarzberg, M.; Frankel, M. *Isr. J. Chem.* **1970**, *8*, 53. (b) Boc-Ala-Pro-OH: $[\alpha]_D^{28}$ –89.9° (EtOH, *c* = 1): McDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 2555.

(10) **Alternative Synthetic Method of Boc-Ala-Leu-OH**: Boc-Ala-OH (6.24 g, 33.0 mmol) in DMF (15 mL), ice-cooled, was coupled with TFA·H-Leu-OPac (30.0 mmol) containing Et_3N (4.17 mL, 30.0 mmol) in DMF (15 mL) ice-chilled using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl; 6.90 g, 36.0 mmol) and 1-hydroxybenzotriazole (HOBt; 6.08 g, 45.0 mmol). The solution was stirred for 6 h in an ice-bath and then for 18 h at r.t. After the treatment of the produced Boc-Ala-Leu-OPac (3.91 g, 9.30 mmol) with Zn powder (19.62 g, 300 mmol) in AcOH (30 mL), the desired product Boc-Ala-Leu-OH (1.13 g, 3.74 mmol) was obtained by the recrystallization from EtOAc–Et_2O–hexane. Boc-Ala-Leu-OH: mp 137.6–137.8 °C. MS (FAB): m/z = 303 $[M + H]^+$, 325 $[M + Na]^+$. t_R = 13.6 min. HPLC conditions: column, Waters μ-Bondasphere (3.9 × 150 mm, C18, 5 μm, and 300 Å); solvent: 5–95% MeCN–H_2O–0.1% TFA; linear gradient, 30 min; flow rate: 1.0 mL/min; and detection, λ = 210 nm; $[\alpha]_D^{22}$ –40.7° (MeOH, *c* = 1.00).