

Convenient Peptide Synthesis Using Unprotected α -Amino Acids Containing Another Hydrophilic Moiety under Basic Conditions

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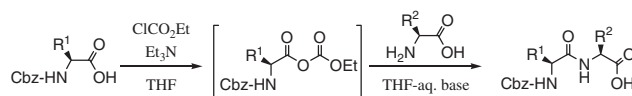
Carboxylic acids **1** and **7** reacted effectively with unprotected α -amino acids **2** containing another hydrophilic moiety under basic conditions via activation by ethyl chloroformate and triethylamine to afford the corresponding amides in 74–99% yields.

Effective coupling reagents for *N*-acylation of unprotected α -amino acids via activation of carboxylic acids have been reported.¹ These types of active species are relatively stable in water and easy to handle. For example, the coupling reactions using unprotected α -amino acids and *N*-acyl derivatives obtained by the combination of 1-hydroxysuccinimide (HOSu) or 1-hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCl) proceed under mild conditions.^{1b} On the other hand, unprotected α -amino acids react easily with *p*-nitrophenyl esters obtained by *p*-nitrophenyl chloroformate (pNPCF) in the presence of triethylamine and 4-dimethylaminopyridine (DMAP), and no racemization was observed.^{1d} However, these types of activating reagents are relatively expensive. We have just reported convenient preparation of dipeptides without protection of *C*-terminals via mixed carbonic carboxylic anhydrides.^{1a}

Herein, we describe condensation of carboxylic acids with unprotected α -amino acids containing hydrophilic groups under basic conditions via activation by ethyl chloroformate and triethylamine (Scheme 1).

In a preliminary investigation, the reaction of 3-phenylpropanoic acid (**1**) with 1.5 equivalents of unprotected α -amino acids **2a–2e** in the presence of 1.4 equivalents of ethyl chloroformate and 3.0 equivalents of triethylamine in tetrahydrofuran (THF)–H₂O afforded the corresponding amide **3a–3e** as indicated in Table 1. The reaction of **1** with unprotected α -amino acids containing an alcohol (**2a**), a thiol (**2b**), a phenol (**2c**), or an amide (**2d**) moiety gave the corresponding products in 73–89% yields within a short reaction time. Small amounts of by-products **4** and **1'** were detected by ¹H NMR analysis of the concentrates of the mother solutions (Entries 1–4). However, the reaction of **1** with L-Glu–OH (**2e**) slightly soluble in water afforded **3e** in only 50% yield with a 48% yield (based on **1**) of the by-product **1'** (Entry 5).

Next, the amidation of 3-phenylpropanoic acid (**1**) with L-Glu–OH (**2e**) under acidic or basic conditions was examined, and the results are summarized in Table 2. The reaction of **1** with **2e** under acidic conditions using 1.5 equivalents of HCl afforded **3e** in 6% yield with a 76% yield of the corresponding carbonic carboxylic anhydride **5** as shown in Entry 1. In contrast, the reaction of **1** with **2e** under basic conditions using 1.5 equivalents of NaHCO₃, NaOH, Na₂CO₃, or K₂CO₃ gave **3e** in 94–99% yields (Entries 3–6). The amidation of **1** with **2e** in the presence of 20 equivalents of K₂CO₃ afforded the amide **3e**



Scheme 1.

Table 1. Amidation of 3-phenylpropanoic acid (**1**) with unprotected α -amino acids **2a–2e**^a

Entry	R	2	Yield of 3 /%		Ratio of 3 : 4 : 1' ^b
			Corrected ^c	Crystal	
1	HOCH ₂	2a	89	87	19:55:26
2	HSCH ₂	2b	86	86	16:71:13
3	<i>p</i> -HOC ₆ H ₄	2c	73	71	10:6:83
4	H ₂ NCOCH ₂ CH ₂	2d	73	73	0:0:100
5	HO ₂ CCH ₂ CH ₂	2e	50	45	13:24:64

^aAll reactions were carried out with 1.0 mmol of **1**, 1.4 mmol of ethyl chloroformate, and 3.0 mmol of triethylamine in 20 mL of THF. After stirring for 30 min at 0 °C, 1.5 mmol of unprotected α -amino acid **2** in 20 mL of H₂O was added at 0 °C to the reaction mixture. ^bDetermined by ¹H NMR analysis of the crude concentrate of the mother solution. ^cCrystal + **3** in the mother solution (**3** + **4** + **1'**).

in 67% yield with a 30% yield (based on **1**) of the by-product **1'** (Entry 7).

A possible pathway is shown in Scheme 2. A carboxylic acid **2** reacted with a mixed carbonic carboxylic anhydride **5** to form the corresponding carboxylic anhydride intermediate **6** or **6-H**⁺ predominantly. Under neutral or basic conditions, the formation of **6** proceeds easily, followed by the intramolecular reaction of **6** via five-membered transition state to afford the corresponding amide, because both the carboxylate anion and free amine are good nucleophiles (Entries 3–6 in Table 2). The reaction does not work well under acidic conditions because the carboxylic acid possesses low nucleophilicity and ammonium ion is not nucleophilic (Entry 1 in Table 2).

Furthermore, condensation of 3-phenylpropanoic acid (**1**) and *N*-benzyloxycarbonyl (Cbz)-L-phenylalanine (**7**) with several kinds of unprotected α -amino acids **2a–2e** in an aqueous NaHCO₃ solution via mixed carbonic carboxylic anhydride are collected in Tables 3 and 4, respectively. The yields of the

Table 2. Effect of additives on the amidation of 3-phenylpropanoic acid (**1**) with L-glutamic acid (**2e**)^a

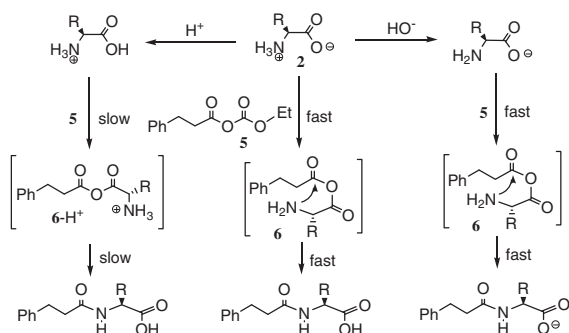
Reaction scheme for Table 2: 3-phenylpropanoic acid (**1**) reacts with L-glutamic acid (**2e**) in the presence of ethyl chloroformate, triethylamine, and THF at 0 °C for 30 min, followed by an additive and water at 0 °C for 30 min, to yield a mixture of products **3e**, **4e**, and **1'**.

Entry	Additive	pH value ^c	Yield of 3e /%		Ratio of 3e : 4e : 1' ^b
			Corrected ^d	Crystal	
1	HCl	1.0	6	6	0:0:0
2	free	4.5	50	45	13:24:64
3	NaHCO ₃	6.5	94	90	22:55:23
4	NaOH	7.5	99	99	7:52:41
5	Na ₂ CO ₃	8.0	98	98	5:27:68
6	K ₂ CO ₃	9.0	98	96	9:39:52
7 ^e	K ₂ CO ₃	12	67	66	6:24:71

^aAll reactions were carried out with 1.0 mmol of **1**, 1.4 mmol of ethyl chloroformate, and 3.0 mmol of triethylamine in 20 mL of THF. After stirring for 30 min at 0 °C, a solution of 1.5 mmol of L-glutamic acid (**2e**) and 1.5 mmol of an additive in 20 mL of H₂O was added at 0 °C to the reaction mixture.

^bDetermined by ¹H NMR analysis of the crude concentrate of the mother solution. ^cThe pH value of the solution of **2e** and an additive in 20 mL of H₂O was measured with pH-test paper.

^dCrystal + **3e** in the mother solution (**3e** + **4e** + **1'**). ^eThe reaction was carried out with 20 mmol of K₂CO₃.

**Scheme 2.** Possible pathway of the amidation via mixed carbonic carboxylic anhydride **5**.

reactions with α -amino acids containing an alcohol (**2a**), a thiol (**2b**), an amide (**2d**), or a carboxylic acid (**2e**) moiety were higher in all cases under the basic conditions (Entries 1, 2, 5, and 6 in Table 3) than those without base (Table 1). The reaction of **1** with L-Tyr-OH (**2c**) slightly soluble in an aqueous NaHCO₃ solution afforded **3c** in 68% yield (Entry 3 in Table 3). L-Tyr-OH (**2c**) is dissolved in an aqueous NaOH solution and the reaction of **1** with **2c** gave the corresponding amide **3c** in 90% yield (Entry 4 in Table 3). Finally, the reaction of **7** with unprotected α -amino acids **2a–2e** under basic conditions afforded the peptides **8a–8f** in 82–95% yields as shown in Table 4. L- and D-Cbz-Phe-OH with L-Glu-OH were converted into the corresponding single diastereomers, which are checked by ¹H NMR analysis, in 93% and 83% yields, respectively

Table 3. Amidation of 3-phenylpropanoic acid (**1**) with unprotected α -amino acids **2a–2e** under basic conditions^a

Reaction scheme for Table 3: 3-phenylpropanoic acid (**1**) reacts with an unprotected α -amino acid (**2**) in the presence of ethyl chloroformate, triethylamine, and THF at 0 °C for 30 min, followed by NaHCO₃ and water at 0 °C for 30 min, to yield a mixture of products **3**, **4**, and **1'**.

Entry	R	2	Yield of 3 /%		Ratio of 3 : 4 : 1' ^b
			Corrected ^c	Crystal	
1	HOCH ₂	2a	93	91	13:57:30
2	HSCH ₂	2b	94	92	11:80:10
3	<i>p</i> -HOC ₆ H ₄	2c	68	66	10:10:80
4 ^d	<i>p</i> -HOC ₆ H ₄	2c	90	79	29:52:19
5	H ₂ NCOCH ₂ CH ₂	2d	74	74	0:0:100
6	HO ₂ CCH ₂ CH ₂	2e	94	90	22:55:23

^aAll reactions were carried out with 1.0 mmol of **1**, 1.4 mmol of ethyl chloroformate, and 3.0 mmol of triethylamine in 20 mL of THF. After stirring for 30 min at 0 °C, a solution of 1.5 mmol of unprotected α -amino acid **2** and 1.5 mmol of NaHCO₃ in 20 mL of H₂O was added at 0 °C to the reaction mixture. ^bDetermined by ¹H NMR analysis of the crude concentrate of the mother solution. ^cCrystal + **3** in the mother solution (**3** + **4** + **1'**). ^dNaOH was used instead of NaHCO₃.

Table 4. Synthesis of dipeptides **8** with Cbz-L-Phe-OH (**7**) and unprotected α -amino acids **2a–2e** under basic conditions^a

Reaction scheme for Table 4: Cbz-L-phenylalanine (**7**) reacts with an unprotected α -amino acid (**2**) in the presence of ethyl chloroformate, triethylamine, and THF at 0 °C for 30 min, followed by NaHCO₃ and water at 0 °C for 30 min, to yield a dipeptide **8**.

Entry	R	2	Dipeptide 8	Yield ^b /%
1	HOCH ₂	2a	8a	95
2	HSCH ₂	2b	8b	91
3 ^c	<i>p</i> -HOC ₆ H ₄	2c	8c	93
4	H ₂ NCOCH ₂ CH ₂	2d	8d	82
5 ^d	HO ₂ CCH ₂ CH ₂	2e	8e	93
6 ^e	HO ₂ CCH ₂ CH ₂	2e	8f	83

^aAll reactions were carried out with 1.0 mmol of Cbz-L-phenylalanine (**7**), 1.4 mmol of ethyl chloroformate, and 3.0 mmol of triethylamine in 20 mL of THF. After stirring for 30 min at 0 °C, a solution of 1.5 mmol of α -amino acid **2** and 1.5 mmol NaHCO₃ in 20 mL of H₂O was added at 0 °C to the reaction mixture. ^bIsolated yield. ^cNaOH was used instead of NaHCO₃. ^dDetails of experimental procedure in ref. 2. ^eThis reaction was carried out with 0.5 mmol of Cbz-D-phenylalanine (**7'**), 0.7 mmol of ethyl chloroformate, and 1.5 mmol of triethylamine in 10 mL of THF. After stirring for 30 min at 0 °C, 0.75 mmol of α -amino acid **2** in 10 mL of H₂O was added at 0 °C to the reaction mixture.

(Entries 5 and 6). These results indicate that racemization does not proceed under the reaction conditions.

In conclusion, we have improved the peptide synthesis using unprotected α -amino acids **2a–2e** containing another hydrophilic moiety under basic conditions to afford the

corresponding dipeptide **8a–8f** in 82–95% yields. Further investigations about this type of condensation for preparation of polypeptide are under way in our group.

References and Notes

- 1 a) T. Noguchi, N. Tehara, Y. Uesugi, S. Jung, N. Imai, *Chem. Lett.* **2012**, *41*, 42, and the references cited therein. b) F. Fujisaki, M. Oishi, K. Sumoto, *Chem. Pharm. Bull.* **2007**, *55*, 124. c) A. R. Katritzky, K. Suzuki, S. K. Singh, *Synthesis* **2004**, 2645. d) P. Gagnon, X. Huang, E. Therrien, J. W. Keillor, *Tetrahedron Lett.* **2002**, *43*, 7717. e) J. Ottl, H. J. Musiol, L. Moroder, *J. Pept. Sci.* **1999**, *5*, 103.
- 2 A typical procedure of the amidation of **7** with **2e** by using ethyl chloroformate is as follows. To a solution of 299 mg (1.0 mmol) of Cbz-L-Phe-OH (**7**) in 20 mL of THF, 134 μ L (1.4 mmol, 1.4 equiv) of ethyl chloroformate and 415 μ L (3.0 mmol, 3.0 equiv) of triethylamine were added at 0 °C.

After stirring for 30 min at 0 °C, a solution of 220 mg (1.50 mmol, 1.5 equiv) of L-Glu-OH (**2e**) in 20 mL of aqueous NaHCO₃ (1.50 mmol, 1.5 equiv) solution was added at 0 °C to the colorless suspension. The mixture was stirred for 30 min at 0 °C, the colorless clear solution was concentrated in vacuo. To the residue was added a 1.0 M aqueous HCl solution to adjust to pH 2. The resulted suspension was extracted with 100 mL of ethyl acetate, and the organic layer was washed with 10 mL of brine, and dried over MgSO₄, and concentrated. The crude product was recrystallized from 80 mL of a 1:7 mixture of EtOAc and hexane to afford 399 mg (93% yield) of **8e** (Cbz-L-Phe-L-Glu-OH). **8e**: colorless powder; ¹H NMR (CD₃OD): δ 1.89–1.99 (1H, m, CH), 2.15–2.33 (1H, m, CH), 2.39 (2H, t, J = 7.5 Hz, CH₂), 2.85 (1H, dd, J = 9.7, 13.9 Hz, CH_A), 3.15 (1H, dd, J = 5.1, 13.9 Hz, CH_B), 4.40–4.47 (2H, m, CH₂), 5.01 (2H, s, CH₂), 7.18–7.32 (10H, m, C₆H₅ $\times$ 2).