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

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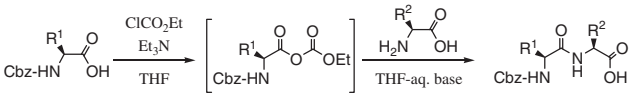
Carboxylic acids **1** and **7** reacted effectively with unprotected α-amino acids **2** containing another hydrophilic moiety under basic conditions via activation by ethyl chloroformate and triethylamine to afford the corresponding amides in 74–99% yields.

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 (WSCI) 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).

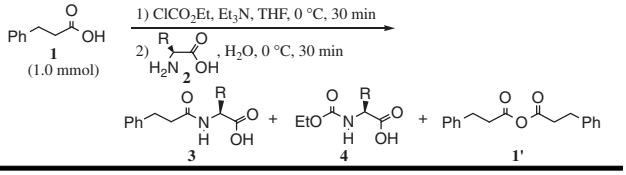
In a preliminary investigation, the reaction of 3-phenylpropanoic acid (**1**) with 1.5 equivalents of unprotected α-amino acids **2a**–**2e** in the presence of 1.4 equivalents of ethyl chloroformate and 3.0 equivalents of triethylamine in tetrahydrofuran (THF)–H_2O afforded the corresponding amide **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 ^{1}H 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 $NaHCO_3$, NaOH, Na_2CO_3, or K_2CO_3 gave **3e** in 94–99% yields (Entries 3–6). The amidation of **1** with **2e** in the presence of 20 equivalents of K_2CO_3 afforded the amide **3e** in 67% yield with a 30% yield (based on **1**) of the by-product **1′** (Entry 7).



Scheme 1.

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



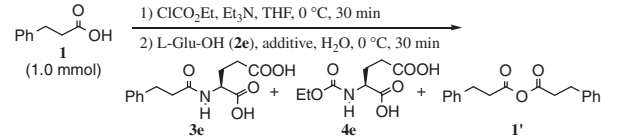
Entry	R	2	Yield of **3**/%		Ratio of **3**:**4**:**1′**[b]
			Corrected[c]	Crystal	Mother solution
1	$HOCH_2$	**2a**	89	87	19:55:26
2	$HSCH_2$	**2b**	86	86	16:71:13
3	*p*-HOC_6H_4	**2c**	73	71	10:6:83
4	$H_2NCOCH_2CH_2$	**2d**	73	73	0:0:100
5	$HO_2CCH_2CH_2$	**2e**	50	45	13:24:64

[a]All 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 H_2O was added at 0 °C to the reaction mixture. [b]Determined by ^{1}H NMR analysis of the crude concentrate of the mother solution. [c]Crystal + **3** in the mother solution (**3** + **4** + **1′**).

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).

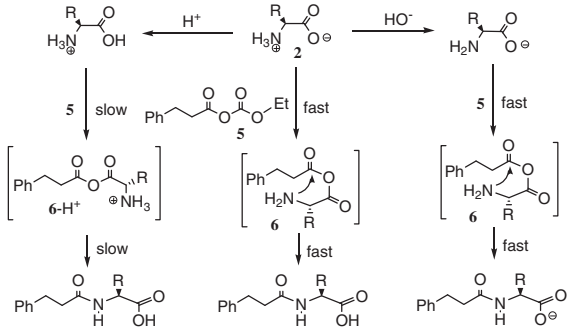
Furthermore, condensation of 3-phenylpropanoic acid (**1**) and *N*-benzyloxycarbonyl (Cbz)-L-phenylalanine (**7**) with several kinds of unprotected α-amino acids **2a**–**2e** in an aqueous $NaHCO_3$ solution via mixed carbonic carboxylic anhydride are collected in Tables 3 and 4, respectively. The yields of the

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



Entry	Additive	pH value[c]	Yield of **3e**/%		Ratio of **3e**:**4e**:**1′**[b]
			Corrected[d]	Crystal	Mother solution
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_2CO_3	8.0	98	98	5:27:68
6	K_2CO_3	9.0	98	96	9:39:52
7[e]	K_2CO_3	12	67	66	6:24:71

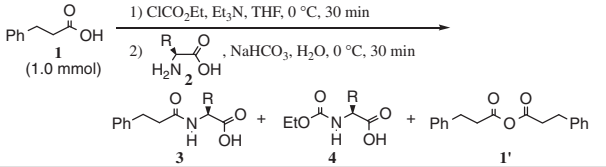
[a]All reactions were carried out with 1.0 mmol of **1**, 1.4 mmol of ethyl chloroformate, and 3.0 mmol of triethylamine in 20 mL of THF. After stirring for 30 min at 0 °C, a solution of 1.5 mmol of L-glutamic acid (**2e**) and 1.5 mmol of an additive in 20 mL of H_2O was added at 0 °C to the reaction mixture. [b]Determined by ^{1}H NMR analysis of the crude concentrate of the mother solution. [c]The pH value of the solution of **2e** and an additive in 20 mL of H_2O was measured with pH-test paper. [d]Crystal + **3e** in the mother solution (**3e** + **4e** + **1′**). [e]The reaction was carried out with 20 mmol of K_2CO_3.



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 $NaHCO_3$ 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 ^{1}H NMR analysis, in 93% and 83% yields, respectively

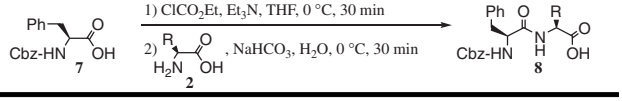
Table 3. Amidation of 3-phenylpropanoic acid (**1**) with unprotected α-amino acids **2a**–**2e** under basic conditions[a]



Entry	R	**2**	Yield of **3**/%		Ratio of **3**:**4**:**1′**[b]
			Corrected[c]	Crystal	Mother solution
1	$HOCH_2$	**2a**	93	91	13:57:30
2	$HSCH_2$	**2b**	94	92	11:80:10
3	*p*-HOC_6H_4	**2c**	68	66	10:10:80
4[d]	*p*-HOC_6H_4	**2c**	90	79	29:52:19
5	$H_2NCOCH_2CH_2$	**2d**	74	74	0:0:100
6	$HO_2CCH_2CH_2$	**2e**	94	90	22:55:23

[a]All 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 $NaHCO_3$ in 20 mL of H_2O was added at 0 °C to the reaction mixture. [b]Determined by ^{1}H NMR analysis of the crude concentrate of the mother solution. [c]Crystal + **3** in the mother solution (**3** + **4** + **1′**). [d]NaOH was used instead of $NaHCO_3$.

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



Entry	R	**2**	Dipeptide **8**	Yield[b]/%
1	$HOCH_2$	**2a**	**8a**	95
2	$HSCH_2$	**2b**	**8b**	91
3[c]	*p*-HOC_6H_4	**2c**	**8c**	93
4	$H_2NCOCH_2CH_2$	**2d**	**8d**	82
5[d]	$HO_2CCH_2CH_2$	**2e**	**8e**	93
6[e]	$HO_2CCH_2CH_2$	**2e**	**8f**	83

[a]All 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 $NaHCO_3$ in 20 mL of H_2O was added at 0 °C to the reaction mixture. [b]Isolated yield. [c]NaOH was used instead of $NaHCO_3$. [d]Details of experimental procedure in ref. 2. [e]This 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 H_2O 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 µL (1.4 mmol, 1.4 equiv) of ethyl chloroformate and 415 µL (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 $NaHCO_3$ (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 $MgSO_4$, 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; ^{1}H NMR (CD_3OD): δ 1.89–1.99 (1H, m, CH), 2.15–2.33 (1H, m, CH), 2.39 (2H, t, $J = 7.5$ Hz, CH_2), 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, CH_2), 5.01 (2H, s, CH_2), 7.18–7.32 (10H, m, $C_6H_5 \times 2$).

