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Convenient Peptide Synthesis without Protection of *C*-Terminals

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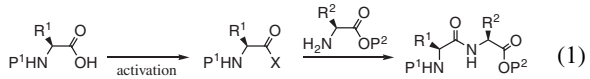
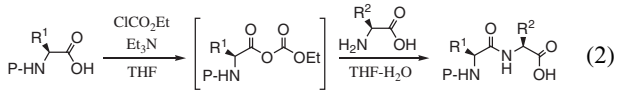
Condensation of carboxylic acids **1** and **5** with unprotected α-amino acids **2** via activation by ethyl chloroformate and triethylamine proceeded effectively to afford the corresponding amides in 50–99% yields. Tripeptide **7c** was obtained in 42% yield from the dipeptide **6c** in a similar manner.

A variety of peptide synthetic methods have been developed for important chemical and biomedical research.[1] For example, activation of carboxylic acids to reactive intermediates such as acyl halides, acyl azides, acyl imidazoles, esters, anhydrides, and mixed carbonic carboxylic anhydrides is necessary for nucleophilic attack by amines. Among them, mixed carbonic carboxylic anhydrides are particularly attractive intermediates because they are easily prepared from the corresponding carboxylic acid, are very stable, and react readily with nucleophiles.[2] The conventional procedure involves activation of the carbonyl group with alkyl chloroformate in the presence of a tertiary amine to form the corresponding mixed carbonic carboxylic anhydride and the reaction of this intermediate with the amine component.



(1)

Herein, we describe condensation of carboxylic acids with unprotected α-amino acids in the presence of ethyl chloroformate and triethylamine.

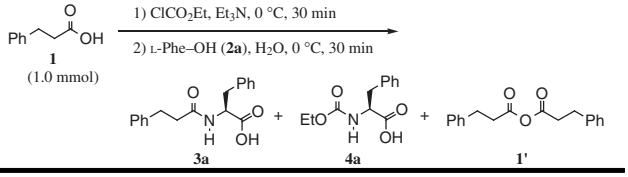


(2)

In a preliminary investigation, the reaction of 3-phenylpropanoic acid (**1**) with 1.5 equivalents of L-phenylalanine (**2a**) 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** in 99% yield as indicated in Entry 2 of Table 1. The solvent effect of the reaction was examined and the results are displayed in Table 1. In the case of using dimethyl sulfoxide (DMSO) as a solvent, poor yield was observed (10% in Entry 4). *N*,*N*-Dimethylformamide (DMF) works as a solvent in the reaction to afford a moderate yield (63% in Entry 5). Amidation of **1** with **2a** proceeded in ether, THF, 1,4-dioxane, acetone, and acetonitrile to give **3a** in excellent yields (93–99%) within a short reaction time except for ether. A small amount of by-products **4a** and **1′** was detected with ^{1}H NMR analysis of the concentrates of the mother solutions in Entries 1–3, 6, and 7 of Table 1.

Next, the effect of the quantity of ethyl chloroformate on the amidation was examined and the results are summarized in Table 2. The carboxylic acid **1** was efficiently coupled with **2a** to afford **3a** in 90–99% yields, and a small amount of by-products **4a** and **1′** was detected with ^{1}H NMR analysis of the

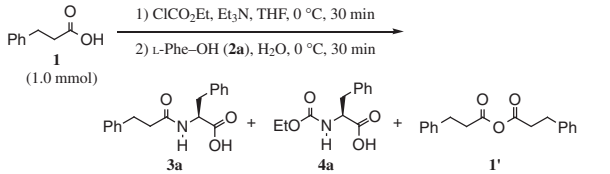
Table 1. Solvent effect on the amidation of 3-phenylpropanoic acid (**1**) with L-phenylalanine (**2a**) in the presence of ethyl chloroformate[a]



Entry	Solvent	Yield of **3a**/%		Ratio of **3a**:**4a**:**1′**[b]
		Corrected[c]	Crystal	Mother solution
1[d]	ether	93	84	27:57:16
2	THF	99	93	24:53:22
3	1,4-dioxane	95	92	14:63:23
4[e]	DMSO	10	10	0:0:100
5[e]	DMF	63	61	9:5:86
6	acetone	95	93	18:54:29
7	acetonitrile	93	90	28:41:31

[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 the solvent. After stirring for 30 min at 0 °C, 1.5 mmol of L-phenylalanine (**2a**) in 20 mL of H_2O was added at 0 °C to the reaction mixtures. [b]Determined by ^{1}H NMR analysis of the concentrates of the mother solutions. [c]Crystal + **3a** of the mother solution (**3a** + **4a** + **1′**). [d]The reaction time was 3 h. [e]The reaction was carried out at room temperature.

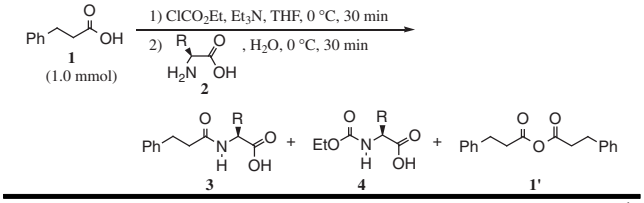
Table 2. Effect of the quantity of ethyl chloroformate on the amidation of 3-phenylpropanoic acid (**1**) with L-phenylalanine (**2a**)[a]



Entry	$ClCO_2Et$ /equiv	Yield of **3a**/%		Ratio of **3a**:**4a**:**1′**[b]
		Corrected[c]	Crystal	Mother solution
1	1.1	90	86	21:44:35
2	1.4	99	93	24:53:22
3	2.0	93	90	8:42:50
4[d]	1.4	96	92	21:59:21
5[e]	1.4	91	86	20:54:25

[a]All reactions were carried out with 1.0 mmol of **1**, 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 L-phenylalanine (**2a**) in 20 mL of H_2O was added at 0 °C to the reaction mixtures. [b]Determined by ^{1}H NMR analysis of the crude concentrates of the mother solutions. [c]Crystal + **3a** of the mother solution (**3a** + **4a** + **1′**). [d]The reaction was carried out from 0 °C to room temperature in both steps. [e]The reaction was carried out from 0 °C to room temperature in the second step.

Table 3. Amidation of 3-phenylpropanoic acid (**1**) with α-amino acids **2a**–**2f** without protection of *C*-terminals[a]



Entry	R	2	Yield of **3**/%		Ratio of **3**:**4**:**1′**[b]
			Corrected[c]	Crystal	Mother solution
1	$PhCH_2$	**2a**	99	93	24:53:22
2	Ph	**2b**	91	84	32:42:26
3	Me_2CH	**2c**	91	89	8:69:23
4	Me_3C	**2d**	90	86	14:73:13
5	$HO_2CCH_2CH_2$	**2e**	50	45	13:24:64
6	$H_2NCOCH_2CH_2$	**2f**	73	73	0:0:100

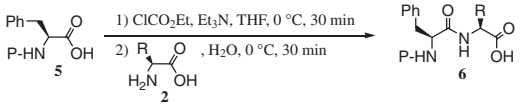
[a]All reactions were carried out with 1.0 mmol of **1**, 1.4 mmol of ethyl chloroformate, and 3.0 mmol of triethylamine in 20 mL of THF. After stirring for 30 min at 0 °C, 1.5 mmol of α-amino acid **2** in 20 mL of H_2O was added at 0 °C to the reaction mixtures. [b]Determined by ^{1}H NMR analysis of the concentrates of the mother solutions in all cases. [c]Crystal + **3** of the mother solution (**3** + **4** + **1′**).

concentrates of the mother solutions in all entries of Table 2. The optimized conditions for preparing **3a** were treatment of **1** with 1.4 equivalents of ethyl chloroformate and 3.0 equivalents of triethylamine in THF at 0 °C, followed by addition of 1.5 equivalents of **2a** in H_2O at 0 °C (Entry 2). Then, Table 3 shows the results of the reactions of 3-phenylpropanoic acid (**1**) with several kinds of α-amino acids **2a**–**2f**. The reactions with α-amino acids **2a**–**2d** afforded the corresponding amides **3a**–**3d** in 90–99% yields (Entries 1–4). Although the reaction of **1** with L-Glu–OH (**2e**) afforded **3e** in 50% yield along with the by-product **1′** of 48% yield based on **1** (Entry 5), the reaction with L-Gln–OH (**2f**), the amide form of **2e**, gave a better yield (73% in Entry 6).

Finally, condensations of N-protected L-phenylalanine **5a**–**5c** with several kinds of unprotected α-amino acids **2a** and **2c**–**2g** via mixed carbonic carboxylic anhydrides are shown in Table 4. We selected *tert*-butyl carbamate (Boc), 9-fluorenylmethyl carbamate (Fmoc), and benzyl carbamate (Cbz) as a protecting group. L-Val–OH protected by the Cbz group worked efficiently to afford the corresponding dipeptide **6c** in 95% yield in comparison with those protected by the Boc and Fmoc groups as shown in Entries 1–3. A bulky α-amino acid **2d** and L-Gln–OH (**2f**) containing amide moiety gave 83% and 93% yields in Entries 4 and 6, respectively, although L-Glu–OH (**2e**) was converted to the dipeptide **6e** in 51% yield as indicated in Entry 5. L- and D-Phe–OH were transformed into the corresponding single diastereomer in 88% yields in Entries 7 and 8, respectively.[3] These results indicate that racemization does not proceed under the reaction conditions. Furthermore, the amidation of Cbz–L-Phe–L-Val–OH (**6c**) with L-Ala–OH was carried out to afford the corresponding tripeptide **7c** in 42% yield.

In conclusion, we have developed a convenient peptide synthesis without protection of *C*-terminals in α-amino acids **2a** and **2c**–**2g** to afford the corresponding dipeptide **6a**–**6h** in 51–

Table 4. Synthesis of dipeptides **6** without protection of *C*-terminals in α-amino acids[a]



Entry	P	5	R	2	Dipeptide 6	Yield[b]/%
1	Boc	**5a**	Me_2CH	**2c**	**6a**	77
2	Fmoc	**5b**	Me_2CH	**2c**	**6b**	83
3	Cbz	**5c**	Me_2CH	**2c**	**6c**	95
4	Cbz	**5c**	Me_3C	**2d**	**6d**	83
5	Cbz	**5c**	$HO_2CCH_2CH_2$	**2e**	**6e**	51
6	Cbz	**5c**	$H_2NCOCH_2CH_2$	**2f**	**6f**	93
7	Cbz	**5c**	$PhCH_2$ (L)	**2a**	**6g**	88
8	Cbz	**5c**	$PhCH_2$ (D)	**2g**	**6h**	88

[a]All reactions were carried out with 1.0 mmol of N-protected L-phenylalanine **5**, 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 α-amino acid **2** in 20 mL of H_2O was added at 0 °C to the reaction mixtures. [b]Isolated yields.

95% yields. No racemization was observed in our method. We still examine further investigations about this type of condensation for preparation of polypeptides.

References and Notes

1 For selected reviews on peptide syntheses: a) M. A. Blaskovich, *Handbook on Syntheses of Amino Acids: General Routes for the Syntheses of Amino Acids*, Oxford University Press, New York, **2010**. b) C. A. G. N. Montalbetti, V. Falque, *Tetrahedron* **2005**, *61*, 10827. c) T. Wieland, M. Bodanszky, *The World of Peptides: A Brief History of Peptide Chemistry*, Springer-Verlag, New York, **1991**, pp. 77–102. For selected reports for peptide syntheses using unprotected α-amino acids: d) P. Gagnon, X. Huang, E. Therrien, J. W. Keillor, *Tetrahedron Lett.* **2002**, *43*, 7717, and the references cited therein. e) M. V. Anuradha, B. Ravindranath, *Tetrahedron* **1997**, *53*, 1123. f) G. W. Anderson, J. E. Zimmerman, F. M. Callahan, *J. Am. Chem. Soc.* **1964**, *86*, 1839.

2 a) W. Chu, Z. Tu, E. McElveen, J. Xu, M. Taylor, R. R. Luedtke, R. H. Mach, *Bioorg. Med. Chem.* **2005**, *13*, 77. b) A. Chaudhary, M. Girgis, M. Prashad, B. Hu, D. Har, O. Repič, T. J. Blacklock, *Tetrahedron Lett.* **2003**, *44*, 5543. c) W.-C. Shieh, J. A. Carlson, M. E. Shore, *Tetrahedron Lett.* **1999**, *40*, 7167. d) R. M. Wenger, *Helv. Chim. Acta* **1983**, *66*, 2672. e) J. Meienhofer, in *Major Methods of Peptide Bond Formation* in *The Peptides: Analysis, Synthesis, Biology*, ed. by E. Gross, J. Meienhofer, Academic Press, New York, **1979**, Vol. 1, pp. 263–314.

3 A typical procedure of the amidation of **5c** using ethyl chloroformate is as follows. To a solution of 299 mg (1.0 mmol) of Cbz–L-Phe–OH (**5c**) in 20 mL of THF, 134 μL (1.4 mmol, 1.4 equivalents) of ethyl chloroformate and 415 μL (3.0 mmol, 3.0 equivalents) of triethylamine were added at 0 °C. After stirring for 30 min at 0 °C, a solution of 248 mg (1.50 mmol, 1.5 equivalents) of L-phenylalanine (**2a**) in 20 mL of H_2O was added at 0 °C to the colorless suspension. The mixture was stirred for 30 min at 0 °C, and the colorless clear solution was concentrated in vacuo. To the residue was added a 1.0 M solution of hydrogen chloride to adjust to pH 2. The resulting suspension was extracted with 100 mL of ethyl acetate, washed with 10 mL of brine, and dried over anhydrous magnesium sulfate. The crude product was recrystallized from 40 mL of an 1:3 mixture of chloroform and hexane to afford 391 mg (88% yield) of **6g** (Cbz–L-Phe–L-Phe–OH). **6g**: colorless powder; ^{1}H NMR ($CDCl_3$): δ 2.98 (1H, dd, J = 6.4, 14.0 Hz, CH_A), 3.02 (2H, d, J = 6.6 Hz, CH_2), 3.14 (1H, dd, J = 6.0, 14.0 Hz, CH_B), 4.38–4.46 (1H, m, CH), 4.74–4.79 (1H, m, CH), 5.06 (2H, s, CH_2), 5.30 (1H, d, J = 7.1 Hz, NH), 6.34 (1H, d, J = 7.3 Hz, NH), 7.02–7.34 (15H, m, C_6H_5 × 3).

