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Dramatic Differences in the Effectiveness among Various Coupling Methods in the Synthesis of Peptides Containing α,α-Diphenylglycine

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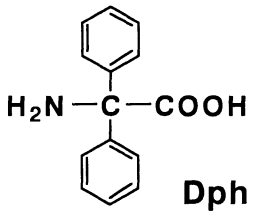
Both couplings of N-benzyloxycarbonyl-α,α-diphenylglycine (Z-Dph) with methyl esters of various amino acids (AA-OMe) and Z-AA with Dph-OMe by various methods can yield Z-Dph-AA-OMe and Z-AA-Dph-OMe, respectively. Yields of the dipeptides remarkably differ from over 90% to 0% depending on the coupling method employed.

Introduction of α,α-disubstituted glycines (DSGs) into peptide chain is of increasing interest in structure-activity studies or conformational studies.[1] However, steric hindrance to coupling can be a severe problem, both at the amino group and the carboxyl group of DSGs.[2] In order to overcome difficulties arising from steric hindrance by DSGs, a variety of methods have been devised. Maia *et al.*[3] could synthesize a tripeptide containing α,α-dibenzylglycine (Dbz) by the modified Ugi reaction, Hardy and Lingham[4] successfully used the oxazolone method for the synthesis of peptides containing α,α-di-*n*-propylglycine (Dpg), and Heimgartner *et al.*[1b] developed the azirin/oxazolone method for the synthesis of various DSG-containing peptides. We also succeeded in synthesizing α,α-diisopropyl glycine (Dip) and its peptides by the modified Ugi reaction at high pressure.[5] Recently, *N*-(9-fluorenylmethoxycarbonyl)-α-aminoisobutyric acid *N*-carboxyanhydride (Fmoc-Aib-NCA) have been successfully used to synthesize Fmoc-$(Aib)_3$-OMe.[6]

Although α,α-diphenylglycine (Dph) is commercially available, Dph-containing peptides so far reported are cyclo$(Dph)_2$ prepared by thermal treatment of 5,5-diphenyl-4-hydroxy-1,2,3-triazole[7] and two dipeptide derivatives with the common sequence Dph-Gly which were synthesized by the oxazolone method in low yields.[8] In this paper we wish to report our findings on syntheses of Dph-containing peptides by using various coupling methods for the reactions [1] and [2].



Z-Dph + AA-OMe ⟶ Z-Dph-AA-OMe [1]
1 **2** **3**

Z-AA + Dph-OMe ⟶ Z-AA-Dph-OMe [2]
4 **5** **6**

In the first place, couplings of Dph with Ala were undertaken by various coupling methods; EDC-HOBt,[9,10] EDC-HOSu,[9,10] mixed anhydride (MA) (using IBCF[9]),[11] DPPA,[9,12] DEPC,[9,13]

Table 1. Synthesis of dipeptides, **3a** and **6a**, containing α,α-diphenylglycine (Dph)[a]

Coupling Method	Z-Dph-Ala-OMe **3a**[b] Time / d	Yield[d] / %	Z-Ala-Dph-OMe **6a**[c] Time / d	Yield[d] / %
EDC-HOBt	3	94[e]	3	2
BOP	1	86	1	43
CMPI-AC9M	1	76	1	74
PyBOP	1	66	1	43
DEPC	1	49	1	15
DPPA	1	33	1	28
EDC-HOSu	3	18	1	0
MA(IBCF)	1	6	1	47
EEDQ	1	0	1	90

a) All the reactions were performed on 0.5 mmol scales, except for the DEPC method (1.0 mmol). b) **3a**, mp 124-125 °C, $[\alpha]_D^{25}$ -4.3° (c 1.0, MeOH). c) **6a**, mp 110-112°C, $[\alpha]_D^{25}$ -28.0° (c 1.0, MeOH). d) Isolated yields. e) Reaction for 1 d also afforded a good yield (81%).

EEDQ,[9,14] BOP,[9,15] PyBOP[9,16] and CMPI-AC9M.[9,17] Both *N*-benzyloxycarbonyl-Dph (Z-Dph)[8] (**1**) and Dph-OMe·HBr (**5**) (mp 199-201 °C) are readily accesible by repeated benzyloxycarbonylation with Z-Cl and by esterification of Z-Dph with diazomethane followed by removal of Z group with HBr/AcOH, respectively.

In the reaction [1], EDC-HOBt, BOP and CMPI-AC9M methods afforded good yields, whereas EEDQ and MA (IBCF) methods scarecely gave products and the PyBOP method gave a lower yield than that with BOP, as shown in Table 1. Generally, reactions giving lower yields afforded a lot of by-products. A typical procedure for the synthesis of Z-Dph-Ala-OMe **3a** is as follows: To a cold solution of Z-Dph (0.50 mmol), Ala-OMe·TosOH (0.60 mmol), HOBt (0.60 mmol), and NMM[9] (0.60 mmol) in DMF (2.0 mL) was added EDC·HCl (0.60 mmol), and the mixture was stirred for 3 d at room temperature. Usual work-up followed by preparative TLC [hexane-EtOAc (3:2)] afforded the desired product **3a** in an excellent yield of 94%. A shorter reaction period (for 1 d) with the EDC-HOBt method also gave a lower but still rather good yield (81%).

In contrast, in the reaction [2] of Ala and Dph, the EEDQ method afforded the best yield, whereas the EDC-HOBt method gave almost no product, and the BOP method only 43% yield, as shown in Table 1. Interestingly, the CMPI-AC9M method gave good yields in both reactions [1] and [2]. A typical procedure for the synthesis of Z-Ala-Dph-OMe (**6a**) was carried out by addition of EEDQ (0.60 mmol) to a cold solution of Z-Ala (0.60 mmol), Dph-OMe·HBr (0.50 mmol), and NMM (0.50 mmol) in DMF (2.0 mL). After the mixture was stirred for 1 d at room temperature, usual work-up followed by preparative TLC [hexane-EtOAc (2:1)] afforded the desired product in an excellent yield of 90%. Thus, remarkable differences in the effectiveness were observed among coupling methods in the reactions [1] and [2] for the synthesis of peptides containing sterically hindered amino acids. Although the reason for such remarkable differences can not be straightforwardly explained, roughly speaking, there seems to be the following tendency: In the reaction [1], the important factors for higher yields may be the reactivity of reagents to form active intermediates of Z-Dph and the pertinent stability of these intermediates, though mixed anhydrides may be too unstable to undergo slow aminolysis. On the other

Table 2. Synthesis of dipeptides, **3** and **6**, containing α,α-diphenylglycine (Dph)[a]

	Z-Dph-AA-OMe **3**[b]		Z-AA-Dph-OMe **6**[b]		
		Yield[c] / % by		Yield[c] / % by	
AA	Product	Method A[d]	Product	Method B[d]	Method C[d]
Ala	**3a**	94	**6a**	90	74
Leu	**3b**	99	**6b**	86	93
Val	**3c**	77	**6c**	55	56
Ile	**3d**	58	**6d**	42	36
Pro	**3e**	68	**6e**	97	78
Aib	**3f**	30	**6f**	19	11
Dph	**3g**	0	**6g**	0	0

a) All reactions were performed on 0.5 mmol scales. b) All products were identified by ^{1}H and ^{13}C-NMR. c) Isolated yield. d) Methods A, B and C are EDC-HOBt (reaction time, 3 d), EEDQ (1 d) and CMPI-AC9M (1 d) methods, respectively.

hand, in the reaction [2], the more reactivity of active intermediates of Z-Ala toward the aminolysis with Dph-OMe may be important to afford higher yields. Thus, EEDQ is superior because it yields a very active mixed anhydride, while IBCF which also yields a very active mixed anhydride seems to be inferior to EEDQ because of the more bulkiness of the intermediate.

Next, we tried the synthesis of dipeptides containing various amino acids as AA by EDC-HOBt, EEDQ, and CMPI-AC9M methods which had been concluded to be effective, as mentioned above. As shown in Table 2, various dipeptides containing Dph and a common α-amino acid could be prepared in excellent to moderate yields, although couplings with more bulky amino acids, such as Val and Ile, generallyt gave lower yields. In the couplings of Dph with DSG, such as Aib or Dph, both Z-Dph-Aib-OMe (**3f**) and Z-Aib-Dph-OMe (**6f**) could be obtained, even though in low yields, but attempts to synthesize a Dph dipeptide failed in any methods.

Further, syntheses of Dph-containing tripeptides, Z-Ala-Dph-Ala-OMe and Z-Val-Dph-Val-OMe, were also tried. Removal of Z group from dipeptides (**3a** and **3c**) by HBr/AcOH gave DphAla-OMe·HBr and Dph-Val-OMe·HBr in moderate yields of 63 and 58%, respectively. Coupling of Z-Ala and Dph-Ala-OMe by the EEDQ method could afford Z-Ala-Dph-Ala-OMe in a fairly good yield (59%), while coupling of Z-Val and Dph-Val-OMe by the same method gave Z-Val-Dph-Val-OMe only in a poor yield of 6%.

In conclusion, although further investigation is necessary for synthesis of peptides containing bulky residues, the present results have disclosed that various Dph-containing peptides can be easily obtained, if the suitable coupling method is selected, and the results stimulate us to prepare a new type of bioactive peptide analogs which contain a bulky, aromatic residue, Dph. It is noteworthy that effectiveness of coupling methods markedly differ depending upon which terminus, NH_2 or COOH, of Dph is coupled with another residue. This may be attributed to that steric factor and electron-withdrawing ability of two phenyl groups on α-carbon atom influence differently between two reactions, because Dph is as a COOH-activated intermediate in the reaction [1] and an amine component in aminolysis in the reaction [2].

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9) Abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole; HOSu, N-hydroxysuccinimide; IBCF, isobutyl chloroformate; DPPA, diphenylphosphoryl azide; DEPC, diethylphosphoryl cyanide; EEDQ, 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; BOP, benzotriazolyl-N-oxytris(dimethylamino)phosphonium hexafluorophosphate; PyBOP, benzotriazolyloxy-tris(pyrrolidino)-phosphonium hexafluorophosphate; CMPI, 2-chloro-1-methylpyridinium iodide; AC9M (Acid Captor 9M), 9-methyl-3,4-dihydro-2H-pyrido(1,2-a)pyrimidin-2-one; NMM, N-methylmorpholine.
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