CHEMISTRY LETTERS, pp. 65-68, 1981.

PEPTIDE SYNTHESIS BY USING PHENYLPHOSPHONIC ESTER AS A COUPLING REAGENT

Yutaka WATANABE, Naoya MORITO, Ken-ichi KAMEKAWA, and Teruaki MUKAIYAMA
Department of Chemistry, Faculty of Science,
The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113

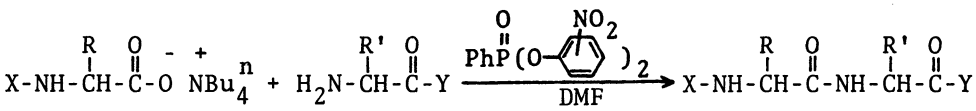
Synthesis of peptides containing various functions was efficiently accomplished by treatment of the tetrabutylammonium salts of N-protected amino acids (or peptides) with amino acid esters in the presence of bis(o- or p-nitrophenyl) phenylphosphonate. Synthesis of leucine-enkephalin was successfully achieved by the above method employing a (3+2) or (4+1) fragment coupling approach.

In a previous paper,[1] it has been pointed out that bis(o- or p-nitrophenyl) phenylphosphonate(1 or 2) is a superior coupling reagent for the peptide synthesis based on the results obtained by the Young test which is known as one of the most severe racemization tests. Moreover, employment of N-protected amino acids as their tetrabutylammonium salts[2] proved to be effective in view of high reactivity and increased solubility, and the reaction was accomplished without use of tertiary amines, which play a main role in causing racemization during coupling reaction.

The results prompted us to apply the method to synthesis of peptides including various functional groups in the side chains. The reaction of tetrabutylammonium salts of N-protected amino acids (or peptides) with amino acid esters in the presence of bis(o- or p-nitrophenyl) phenylphosphonate was carried out according to the following general procedure. A methanol solution of equimolar amounts of an N-protected α-amino acid and tetrabutylammonium hydroxide was subjected to evaporation and the residue was azeotroped with benzene and dried in vacuo. To a stirred DMF (10 ml/mmol) solution of the ammonium salt thus obtained and an α-amino acid ester (1.1 or 0.9 equiv.) was added bis(o- or p-nitrophenyl) phenylphosphonate (1.1 equiv.) and the mixture was stirred under the reaction conditions shown in Table. After removal of DMF in vacuo, the residue was dissolved in ethyl acetate, and the organic solution was washed successively with 2N hydrochloric acid, water (twice), saturated sodium hydrogen carbonate, water(twice), and saturated brine, and then dried ($MgSO_4$). After evaporation of ethyl acetate, the peptide was purified by column or thin layer chromatography on silica gel.

The results are summarized in the table. No protection of functional groups in the side chains such as aliphatic and phenolic hydroxyl groups, and imidazole and indole rings was demonstrated in the present peptide synthesis. The amino acids containing methyl- and benzylthio, nitroguanidino groups did not show any trouble. Further, the carbamoyl group in the side chain of Z-L-asparagine was

Table. Preparation of peptides

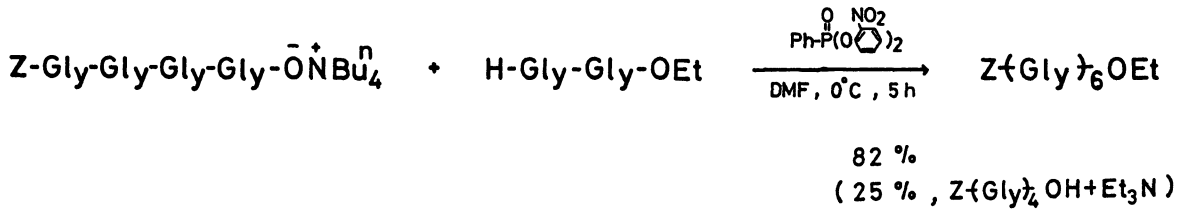


Peptide	Phosphonate	Reaction conditions	Yield (%)	Mp(°C) [lit.]	$[\alpha]_D$(temp.,c,solv.) [lit.]	Ref.[a]
Z-Phe-Ser-OMe[b]	2	0°C,7h then r.t.,6h	73	122-3 [125]	-5.7(23,1.0,DMF) [-5.7]	3
Z-Tyr-Gly-OEt	2	0°C,14h then r.t.,2h	93	168-70 [170-1]	-23.6(25,5.0,DMF) [24.2]	4
Z-Ile-His-OMe	2	0°C,6.5h then r.t.,13h	79	183-4 [181-3]	-44.3(26,1.0, MeOH-NHCl(1:1)) [-44.7]	5
Boc-Trp-Gly-OEt[c]	2	0°C,6h then r.t., 14h	96	111-2 [112-3]	-17.7(25,1.0,DMF) [-18]	6
Z-Asn-Gly-OEt[d]	2	0°C,13h then r.t.,4h	86	185.5-6.5 [185-7]	-5.4(25,1.1,DMF) [-5.6]	7
Z-Met-Gly-OEt	2	0°C,8h then r.t.,1.5h	94	94.5-5 [98-9]	-18.3(24,4.8,EtOH) [-17.9]	8
Z-Cys(Bzl)-Leu-OBzl	1	0°C,8h then r.t.,16.5h	86	85-7	-34.8(25,2.1,DMF)	
Z-Arg(NO_2)-Gly-OEt	2	0°C,13h then r.t.,7h	90	110-1 [119-20]	-10.6(24,2.0,MeOH) [-15.4]	7
Z-Gly-Arg(NO_2)-OBzl	1	0°C,6h then r.t.,15h	82	133-4	-13.0(26,1.0,DMF)	
Z-Tyr-Arg(NO_2)-OBzl	2	0°C,16h then r.t.,5h	73			
Z-Gly-Pro-OBzl	2	0°C,14h then r.t.,5h	85	oil	not messured	
Z-Gly-Phe-Gly-OEt[e]	1	-10°C,7h,0°C,12h then r.t.,2h	81	117.5-8 [118-9]	-12.7(23,2.0,EtOH)] [-12.4(23,2.0,EtOH)]	9

a) Literature values of melting points and specific optical rotations were cited from the same literature indicated by reference number.
b) Other literature values; mp 122-123°C, [α] -6.4(c1,DMF): R. Appel and L. Willms, Chem. Ber., 112, 1057 (1979).
c) Other literature values; mp 118-119°C, $[\alpha]_{25}^{20}$-12.8 (c 1.1,EtOH): Reference 7.
d) Other literature values; mp 188-190°C, $[\alpha]^{25}$-6.0 (c 1.1,DMF): Reference 7.
e) The dotted line indicates the point of coupling.

kept intact during the reaction with ethyl glycinate. According to the present method, the Anderson test gave good result as well as the Young test reported previously.[1] Thus, racemization-free N-benzyloxycarbonylglycyl-L-phenylalanylglycine ethyl ester was prepared by the reaction of tetrabutylammonium salt of N-benzyloxycarbonylglycyl-L-phenylalanine with glycine ethyl ester in the presence of bis(o-nitrophenyl) phenylphosphonate(1).

In order to demonstrate the usefulness of employment of tetrabutylammonium salt, we examined the reaction of glycylglycine ethyl ester(3) and N-benzyloxycarbonylglycylglycylglycylglycine(4) which is hardly soluble in ordinary organic solvents even in DMF. Thus, the reaction of 3 with free 4 in the presence of triethylamine and phosphonate 1 at 0°C for 5 h resulted in the formation of fully protected hexaglycine 5 only in 25% yield. In contrast to the poor result,

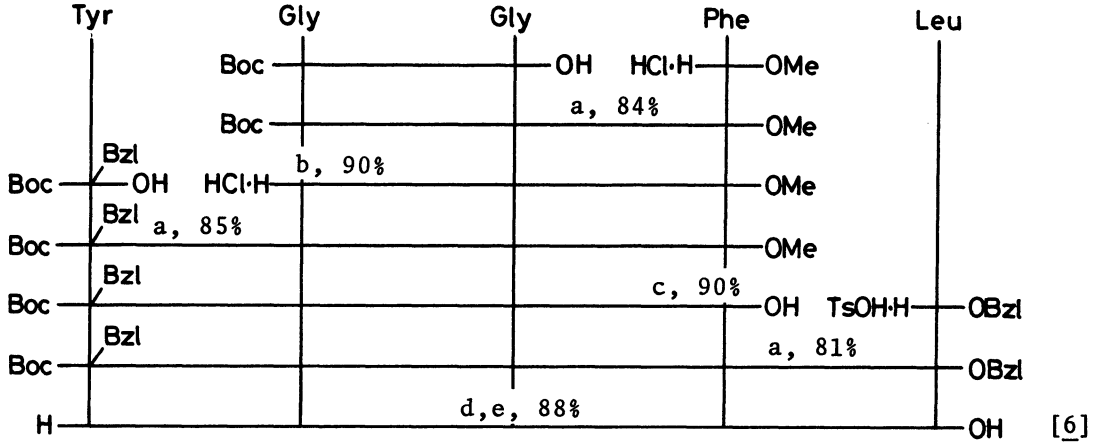


employment of the tetrabutylammonium salt of 4, which easily dissolved in DMF, brought about the smooth reaction under the same reaction conditions to afford 5 in 82% yield. These results clearly indicate that employment of the tetraalkylammoniumsalts increases both the solubility[10] and nucleophilicity of the carboxyl component in the peptide synthesis. Consequently, it is expected that various peptides including large peptides would be smoothly prepared by the present method even in the cases using carboxyl components sparingly soluble in organic solvents.

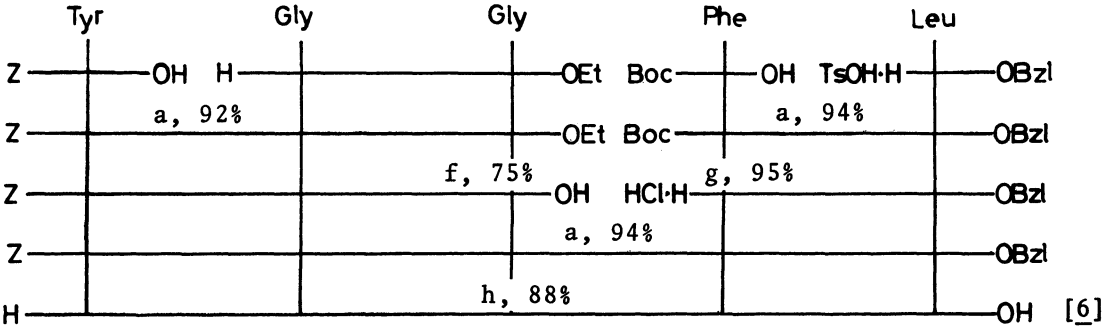
The present method was efficiently applied to synthesis of leucine-enkephalin (6) by means of a (4+1) or (3+2) fragment coupling approach as shown in the

Scheme. Synthesis of leucine-enkephalin

(i) (4 + 1) Fragment coupling



(ii) (3 + 2) Fragment coupling



a, n-$Bu_4\overset{+}{N}\bar{O}H$/MeOH then 1/$Et_3N$/DMF; b, 2.5N HCl/AcOEt; c, 1N NaOH/MeOH/H_2O; d, 5% Pd-C/$A\bar{c}OH$; e, 2.5N HCl/dioxane; f, 1N NaOH/DMF; g, 5N HCl/AcOEt; h, 5% Pd-C/90% aq. AcOH/0.2N HCl(1 equiv).

following scheme. No requirement for protection of the phenolic hydroxyl group in the tyrosine residue was demonstrated in the (3+2) fragment condensation (Scheme ii). Both pentapeptides 6 so obtained were identical chromatographically with an authentic leucine-enkephalin. The melting point and optical rotation found for 6[15] obtained by a (4+1) fragment coupling approach were in accord with those for 6[16] obtained by a (3+2) fragment coupling approach. Furthermore, the activity of each synthetic Leu-enkephalin(6) as an opioid agonist in mouse vas deferens was identical with that of an authentic sample (Protein Research Foundation, Osaka).

Acknowledgement: We wish to thank Dr. Masaaki Ueki and Dr. Shigeru Ikeda, Science University of Tokyo, for gift of an authentic sample of Leu-enkephalin and for amino acid analysis. We are also grateful to Prof. Tetsuo Oka, Tokai University, for the biological activity measurements.

References and Notes

1) T. Mukaiyama, N. Morito, and Y. Watanabe, Chem. Lett., 1979, 1305.
2) Very recently, tetrabutylammonium salt was utilized in amide and peptide syntheses using o-phenylene phosphorochloridate as a coupling reagent: M. Wakselman and F. Acher, Tetrahedron Lett., 21, 2705 (1980).
3) R. A. Boissonnas, St. Guttmann, and P.-A.Jaquenoud, Helv. Chim. Acta, 43, 1349 (1960).
4) K. Okamoto and S. Shimamura, Yakugaku Zasshi, 93, 333 (1973).
5) D. S. Jones, J. Chem. Soc., Perkin Trans. 1, 1972, 1407.
6) M. Chorev and Y. S. Klausner, J. Chem. Soc., Chem. Commun., 1976, 596.
7) T. Mukaiyama, R. Matsueda, and M. Suzuki, Tetrahedron Lett., 1970, 1901.
8) T. Shioiri and S. Yamada, Chem. Pharm. Bull., 22, 859 (1974).
9) M. Goodman and K. C. Stueben, J. Am. Chem. Soc., 81, 3980 (1959).
10) Luisi et al. have been recently reported that proteins such as α-chymotrypsin and trypsin are trasformed from an alkaline solution into cyclohexane in the presence of methyltrioctylammonium chloride: P. L. Luisi, F. J. Bonner, A. Pellegrini, P. Wiget, and R. Wolf, Helv. Chim. Acta, 62, 740 (1979).
11) The specific optical rotation value ($[\alpha]_D^{24}$ +3.9°(c 2, AcOH) of our sample is much different from the reported value ($[\alpha]_D$ +6.5 (c 2, AcOH)(ref. 12). However, $[\alpha]_D$ of the compound prepared according to Bergmann's procedure (Z-Tyr-$NHNH_2$ + H-Gly-Gly-OEt)(ref. 13) showed the same value with that of our sample. Further, the optical rotation, $[\alpha]_D^{25}$ -21.5 (c 1, DMF) of the hydrazide derived from Z-Tyr-Gly-Gly-OEt obtained by our method agreed with the literature value ($[\alpha]_D^{25}$ -21.86 (c 1, DMF)(ref. 14).
12) G. W. Anderson, J. Blodinger, and A. D. Welcher, J. Am. Chem. Soc., 74, 5309 (1952).
13) M. Bergmann and J. S. Fruton, J. Biol. Chem., 118, 405 (1937).
14) S. Wang, B. F. Gisin, D. P. Winter, R. Makofske, and I. D. Kulesha, J. Org. Chem., 42, 1289 (1977).
15) The product was isolated by the use of a silica gel 60 pre-packed column ($CHCl_3$: MeOH:aq. NH_4OH=24:6:1) and then a Sephadex LH-20 column(MeOH). Further, the chromatographically pure pentapeptide was recrysted from methanol, mp 159-161°C [lit.(ref. 17), 158-160°C]; $[\alpha]_D^{26}$+22.5°(c 0.5, 3% aq. AcOH)[lit.(ref. 18)$[\alpha]_D^{24}$ +18.0°(c 0.7, 3% AcOH)]. Found: C, 57.99; H, 6.62; N, 12.04, Calcd. for $C_{28}H_{37}N_5O_7 \cdot 3/2\ H_2O$: C, 57.72; H, 6.92; N, 12.02. Amino acid analysis (after acidic hydrolysis): Tyr, 1.04; Gly, 2.00; Phe, 1.00; Leu, 0.96.
16) The product was isolated by a procedure similar to that employed in a (4+1) fragment coupling approach (ref. 15), mp 157-159°C; $[\alpha]_D^{26}$+22.5°(c 0.75, 3% aq. AcOH). Found: C, 57.77; H, 6.80; N, 12.31. Calcd. for $C_{28}H_{37}N_5O_7 \cdot 3/2\ H_2O$: C, 57.72; H, 6.92; N, 12.02. Amino acid analysis (after acidic hydrolysis): Tyr, 0.97; Gly, 1.97; Phe, 1.04; Leu, 1.02.
17) M. Ueki, T. Inazu, and S. Ikeda, Bull. Chem. Soc. Jpn., 52, 2424 (1979).
18) H. Yajima, H. Ogawa, N. Fujii, and S. Funakoshi, Chem. Pharm. Bull., 25, 740 (1977).

(Received November 15, 1980)