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Amino Acids and Peptides. I. Syntheses of *p*-Methoxybenzyl *p*-Nitrophenyl Carbonate, *p*-Methoxybenzyl Carbazate, and *p*-Methoxybenzyloxycarbonyl Amino Acids[1] with *p*-Methoxybenzyl Chloroformate

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A new method for the syntheses of *p*-methoxybenzyl *p*-nitrophenyl carbonate, *p*-methoxybenzyl carbazate, and *p*-methoxybenzyloxycarbonyl amino acids has been devised. These compounds were obtained in satisfactory yield from sodium *p*-nitrophenolate, 85% hydrazine hydrate, and several amino acids with *p*-methoxybenzyl chloroformate prepared from anisyl alcohol and phosgene in the presence of dimethylaniline in dry ether at −5—−10°C. This direct *p*-methoxybenzyloxycarbonylation of amino acid with the chloroformate is more convenient than the usual Weygand method.

The *p*-methoxybenzyloxycarbonyl [Z(OMe)][2] group has been widely used as a blocking substituent of the amino moiety of amino acids in peptide synthesis.[3,4] This group allows an easier removal by trifluoroacetic acid than the parent group (carbobenzoxy)[5] without the methoxy substituent. *p*-Methoxybenzyloxycarbonyl amino acids[6] were obtained for the first time by the reaction of isocyanocarboxylic acid esters with anisyl alcohol and subsequent hydrolysis. Later Weygand and Hunger[7] prepared these compounds in a synthesis analogous to that of the *t*-butyloxycarbonyl amino acids.[8] The Z(OMe) group was introduced with the use of *p*-methoxybenzyl *p*-nitrophenyl carbonate (II) or *p*-methoxybenzyloxycarbonyl azide prepared by the reaction of *p*-methoxybenzyl carbazate (III) with nitrous acid. Their method has been used in many cases, but the synthesis of II and III involves many steps, and the yields are not so high.

Recently, Schnabel *et al.*[9,10] reported the syntheses of *p*-methoxybenzyloxycarbonyl amino acids with *p*-methoxybenzyl fluoroformate. Jones and Young[11,12] described a method for synthesizing *p*-methoxybenzyl-1-piperidyl carbonate by the use of *p*-methoxybenzyl chloroformate (I). In this method I was prepared from anisyl alcohol and phosgene in dry ether at 20°C, and was coupled with 1-hydroxypiperidine in dry ether at 0°C. For the preparation of *p*-methoxybenzyloxycarbonyl

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[1] S. Sōfuku, M. Mizumura and A. Hagitani, This Bulletin, **42**, 278 (1969).

[2] Abbreviated designation by IUPAC-IUB comission on biochemical nomenclature; *Biochem. J.*, **102**, 203 (1967).

[3] M. Bodansky and M. A. Ondetti, "Peptide Synthesis," John Wiley & Sons, Inc., New York (1966), p. 31.

[4] E. Schröder and K. Lübke, "The Peptide," Vol. 1, Academic Press, New York (1965), p. 34.

[5] M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

[6] F. C. Mckay and N. F. Albertson, *J. Amer. Chem. Soc.*,**79**, 4686 (1957).

[7] F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).

[8] G. W. Anderson and A. C. McGregor, *J. Amer. Chem. Soc.*, **79**, 6180 (1957).

[9] E. Schnabel, H. Herzog, P. Hofmann, E. Klauke and I. Ugi, *Angew. Chem.*, **80**, 396 (1968).

[10] E. Schnabel, H. Herzog, P. Hoffmann, E. Klauke and I. Ugi, *Ann. Chem.*, **716**, 175 (1968).

[11] J. H. Jones and G. T. Young, *Chem. Ind.* (London), **1966**, 1722.

[12] J. H. Jones and G. T. Young, *J. Chem. Soc.*, *C*, **1968**, 53.

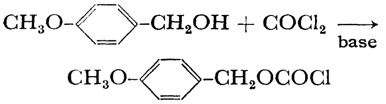
amino acid, *p*-methoxybenzyl-1-piperidyl carbonate and amino acid ester hydrochloride were stirred with sodium acetate in dioxane at 20°C for 24 hr. After the publication of our short communication[1] Honda *et al.*[13] reported a convenient method of synthesis of *p*-methoxybenzyloxycarbonyl amino acids and *p*-methoxybenzyl carbazate with the use of *p*-methoxybenzyl chloroformate.

In the present investigation, I could be prepared readily from anisyl alcohol (1 eq.) and phosgene (3 eq.) in the presence of dimethylaniline (1 eq.) in dry ether at below 0°C. This reagent was very useful for the preparation of *p*-methoxybenzyl *p*-nitrophenyl carbonate (II)[14] and *p*-methoxybenzyl carbazate (III).[15]

p-Methoxybenzyloxycarbonyl amino acids could be prepared directly from a number of amino acids and I under the conditions of the Schotten-Baumann reaction without any accompanying racemization.

Results and Discussion

Reagent I was prepared by the following reaction:



(A)

(I)

Though reaction (A) could take place without a base, its addition was better for the removal of hydrogen chloride from the reaction solution. Because of the instability of I, the amount of phosgene required and the effectiveness of the base used for the preparation of I were examined by comparing the yields of II in combination with the reactions (A) and (B). Among the hydrochlorides of the bases, the one of dimethylaniline was most easily filtered off.

Addition of three equivalents of phosgene to anisyl alcohol in the presence of one equivalent of dimethylaniline gave the best yield of product II, as shown in Table 1.

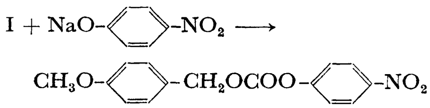
Table 1. Results of the preparation of *p*-methoxybenzyl *p*-nitrophenyl carbonate in xylene

Base used in the preparation of *p*-methoxybenzyl chloroformate*2	Equiv. of phosgene to anisyl alcohol	Yield %
Pyridine	2	19
Quinoline	2	34
Dimethylaniline	2	40
Dimethylaniline	3	66
Dimethylaniline	3	44*1
——	3	48*2

*1 Ether used as solvent

*2 Reaction temperature: 20°C

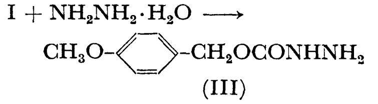
After the completion of reaction (A), it was necessary to remove the salt completely by filtration and excess phosgene by evaporation under a reduced pressure at below 0°C. I was immediately dissolved in a suitable solvent, and used for succeeding reactions. The temperature of the reaction mixture was maintained as low as possible to prevent the degradation of I. For the preparation of II, sodium *p*-nitrophenolate dried by azeotropic dehydration with xylene was used.



(B)

(II)

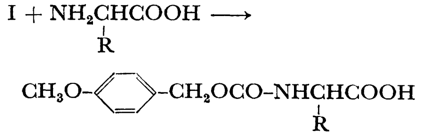
In the preparation of III, it was not necessary to use anhydrous hydrazine. It could be obtained with 85% hydrazine hydrate[16] in a satisfactory yield.



(C)

(III)

Preparation of *p*-methoxybenzyloxycarbonyl amino acids was carried out with two equivalents of I and four equivalents of sodium hydroxide for an amino acid in a mixture of water and tetrahydrofuran (5 : 1).



(D)

In the case of L-serine, carbobenzoxylation of the amino group has been devised,[17] and one equivalent of I was added. The crude product contained only a small amount of *N*,*O*-di-Z(OMe)-L-serine, which was detected by thin layer chromatography (solvent; chloroform : methanol : acetic acid=95 : 5 : 3)[18];

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[13] I. Honda, M. Naruse, M. Kaneoka and S. Sakakibara, the 6th Symposium on Peptide Chemistry of Japan, Fukuoka (November, 1968).

[14] S. Sōfuku, M. Mizumura and A. Hagitani, the 21st Annual Meeting of the Chem. Soc. of Japan, Osaka (April, 1968).

[15] S. Sōfuku, M. Mizumura and A. Hagitani, reported in a short communication to *Nippon Kagaku Zasshi*, **89**, 721 (1968).

[16] "Organikum. Organisch-chemisches Grundpraktikum," Veb Deutscher Verlag der Wissenschaften, Berlin (1965), p. 625.

[17] J. A. Moore, J. R. Dice, E. D. Nicolaides, R. D. Westland and E. L. Wittle, *J. Amer. Chem. Soc.*, **76**, 2884 (1954).

[18] By a private letter from Dr. S. Sakakibara (Inst. for Protein Research, Osaka Univ.). The R_f values refer to the thin layer chromatography with Wakogel B-O. Compounds were detected by spraying them with *ca.* 47% hydrobromic acid and then ninhydrin.

TABLE 2. RESULTS OF THE PREPARATION OF *p*-METHOXYBENZYLOXYCARBONYL AMINO ACIDS

Compound	Yield %	Mp °C	$[\alpha]_D$	Solvent (c 2.0)	Temp. °C
Z(OMe)-glycine	80.0	95—98			
		95—98^{*3}			
Z(OMe)-L-alanine	65.4	78—81	-11.5	acetic acid	22
		74—75^{*1}	-11.9^{*1}		
Z(OMe)-L-aspartic acid	40.9	125—127.5	$+10.3$	acetic acid	18
		122.5—124^{*1}	$+7.1^{*1}$		
Z(OMe)-L-glutamic acid	46.1	109—112	-5.6	acetic acid	22
		109—111^{*1}	-7.44^{*1}		
Z(OMe)-L-serine	53.3	94—96.5	$+6.5$	acetic acid	20
		97—98^{*1}	$+7.1^{*1}$		
		92—94^{*2}	$+5.8^{*2}$		
Z(OMe)-L-valine DCHA	79.2	162—165	$+3.3$	methanol	20
		162—166^{*4}	$+2.8^{*4}$		
Z(OMe)-L-leucine DCHA	88.7	159—162	-6.0	methanol	21
		162^{*1}	-6.67^{*1}		
Z(OMe)-L-phenylalanine DCHA	75.1	159—162	$+22.7$	methanol	21
		157—159^{*1}	$+22.6^{*1}$		
Z(OMe)-L-proline DCHA	82.6	145—147	-23.1	methanol	20
		147.5—149^{*1}	-25.1^{*1}		
Z(OMe)-L-methionine DCHA	87.9	150—153	$+6.0$	methanol	21
		152—153^{*1}	$+3.35^{*1}$		
α-*N*-Z(OMe)-δ-*N*-Z-L-ornithine DCHA	33.4	133—135	$+6.3$	methanol	19
		121—122^{*5}	-4.0^{*5}		

Values, ^{*1} from Ref. 7, ^{*2} from Ref. 10; [α] value at 578 mμ, ^{*3} from Ref. 11, ^{*4} from Ref. 12 and ^{*5} from Ref. 22.

DCHA: dicyclohexylammonium salt, Z(OMe): *p*-methoxybenzyloxycarbonyl, Z: benzyloxycarbonyl.

All melting points are uncorrected.

N-Z(OMe)-L-serine was purified by recrystallization. As shown in Table 2, some products were obtained as the salts of dicyclohexylamine.[19] The *p*-methoxybenzyloxycarbonyl amino acids were recrystallized from ethyl acetate - petroleum ether, and the dicyclohexylammonium salts from ethanol - petroleum ether.

Each derivative gave one spot in thin layer chromatography[18] and was examined by infrared spectrum for –O–CO–N group.[20,21]

Experimental[23]

***p*-Methoxybenzyl Chloroformate (I).** Phosgene (21 m*l*, 0.3 mol) was measured into a glass trap which had been cooled in a dry ice-acetone jar, and poured into a 500-m*l* two-necked conical flask containing 100 m*l* of dry ether, and cooled in an ice-salt bath. A solution of 12.4 m*l* of anisyl alcohol (0.1 mol) in 100 m*l* of dry ether was added in portions to the above solution over a period of 10 min. Then a solution of 12.7 m*l* of dimethylaniline (0.1 mol) in 100 m*l* of dry ether was added dropwise over a period of 1 hr, and the mixture was stirred for 2 hr at -5—-10°C. After filtration of the dimethylaniline hydrochloride formed in the reaction, the filtrate was evaporated under reduced pressure at below 0°C. Twice more 100 m*l* of dry ether was added to the oily residue and evaporation was repeated to remove excess phosgene completely. The residue was immediately dissolved in a suitable solvent and the solution was used for the following reactions.

***p*-Methoxybenzyl *p*-Nitrophenyl Carbonate (II).** To a solution of 0.1 mol of I in 300 m*l* of dry xylene, 17.7 g of powdered dry sodium *p*-nitrophenolate (0.11 mol) was added in portions over a period of 10 min with vigorous stirring at 0—-5°C. After 3 hr at this temperature, it was stirred overnight at room temperature and for 1 hr at 30°C. Excess sodium *p*-nitrophenolate and sodium chloride were filtered off, and the filtrate was washed successively with 0.5N hydrochloric acid, N sodium carbonate, and water, and then dried over anhydrous sodium sulfate. The filtered solution was evaporated *in vacuo*. Recrystallization of the residue from ethanol gave the pure product. Yield, 20.0 g (66.0%); mp, 104.5—105.5°C (lit,[7] 96—97°C). IR spectrum (KBr): 1760 cm^{-1}.

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[19] E. Klieger, E. Shröder and H. Gibian, *Ann. Chem.*, **640**, 157 (1961).

[20] K. Nakanishi, "Infrared Absorption Spectra: Qualitative Analysis," Nanko-do, Tokyo (1960).

[21] L. J. Bellamy, "The Infra-red Spectra of Complex molecules," Chapter 12, Methuen & Co., London (1958).

[22] M. Waki and N. Izumiya, This Bulletin, **41**, 1909 (1968).

[23] The infrared spectra were recorded on a Shimadzu Model IR 27-B Spectrophotometer.

Found: C, 59.52; H, 4.43; N, 4.73%. Calcd for $C_{15}H_{13}NO_6$: C, 59.41; H, 4.32; N, 4.62%.

***p*-Methoxybenzyl Carbazate (III).** A solution of 0.1 mol of I in 100 m*l* of dry ether was added in portions over a period of 10 min to a mixture of 29.4 g of 85% hydrazine hydrate (0.5 mol) and 50 m*l* of ether with vigorous stirring at -30—-60°C. A white precipitate was immediately formed. After 30 min, 300 m*l* of water was added and the mixture was filtered. The precipitate was thoroughly washed with water. The amount of this precipitate was two-thirds of the total product, and the other one-third was isolated from the ether layer. The combined product was recrystallized from ethyl acetate-petroelum ether. Yield, 15.0 g (76.5%); mp, 74—75°C (lit,[7] 71—74°C). IR spectrum (KBr): 1690, 3330 cm^{-1}.

Found: C, 55.36; H, 6.31; N, 14.32%. Calcd for $C_9H_{12}N_2O_3$: C, 55.09; H, 6.16; N, 14.28%.

***p*-Methoxybenzyloxycarbonylglycine.** A solution of 0.1 mol of I in 70 m*l* of tetrahydrofuran was added in portions over a period 10 min to a solution of 3.75 g of glycine (0.05 mol) in 200 m*l* of N sodium hydroxide containing 40 m*l* of tetrahydrofuran with vigorous stirring at 0—5°C. After 2 hr under these conditions, the reaction mixture was washed with 100 m*l* of ether, and acidified with 21 g of citric acid (0.1 mol) at 0—5°C, and the product was extracted with 500 m*l* of ethyl acetate. The organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The resulting residue was crystallized from ethyl acetate and petroleum ether as fine crystalline plates; yield, 9.57 g. IR spectrum (KBr): 1695 cm^{-1}.

Found: C, 55.14; H, 5.52; N, 6.00%. Calcd for $C_{11}H_{13}NO_5$: C, 55.24; H, 5.48; N, 5.86%.

***p*-Methoxybenzyloxycarbonyl-L-alanine.** By the same method as for Z(OMe)-glycine, 8.32 g of rod crystals of pure product was obtained from 4.45 g of L-alanine (0.05 mol) and 0.1 mol of I. IR spectrum (KBr): 1690 cm^{-1}.

Found: C, 56.94; H, 5.83; N, 5.70%. Calcd for $C_{21}H_{15}NO_5$: C, 56.90; H, 5.98; N, 5.54%.

***p*-Methoxybenzyloxycarbonyl-L-aspartic Acid.** To a solution of 6.65 g of L-aspartic acid (0.05 mol) in 1.5N sodium hydroxide (200 m*l*) containing tetrahydrofuran (40 m*l*), 0.1 mol of I in tetrahydrofuran (70 m*l*) was added in portions over a period of 10 min at 0—5°C. The mixture was stirred for 2 hr at this temperature, then washed with ether (100 m*l*), and acidified with 35 g of citric acid; the product was extracted with ethyl acetate (500 m*l*). The organic layer was washed with water and dried over anhydrous sodium sulfate. The solution was concentrated under reduced pressure, and recrystallization of the residue from ethyl acetate-petroleum ether gave a pure granular product. Yield, 6.07 g. IR spectrum (KBr): 1710 cm^{-1}.

Found: C, 52.54; H, 5.25; N, 4.84%. Calcd for $C_{13}H_{15}NO_7$: C, 52.34; H, 5.41; N, 4.70%.

***p*-Methoxybenzyloxycarbonyl-L-glutamic Acid.** By the same method as for Z(OMe)-L-aspartic acid, the crude product was obtained from 7.36 g of L-glutamic acid (0.05 mol) and 0.1 mol of I, and crystallized as pure granules (7.20 g) from ethyl acetate and petroleum ether. IR spectrum (KBr): 1710 cm^{-1}.

Found: C, 53.76; H, 5.22; N, 4.58%. Calcd for $C_{14}H_{17}NO_7$: C, 54.00; H, 5.50; N, 4.50%.

***p*-Methoxybenzyloxycarbonyl-L-serine (A).** To a solution of 10.5 g of L-serine (0.1 mol) in a mixture of N sodium hydroxide (200 m*l*) and 40 m*l* of tetrahydrofuran, a solution of 0.1 mol of I in 70 m*l* of tetrahydrofuran was added in portions over a period of 10 min with vigorous stirring at 0—5°C. The reaction mixture was stirred at this temperature for 2 hr, and then washed with 100 m*l* of ether, and acidified with 21 g of citric acid at 0—5°C. An oily product was extracted with ethyl acetate. The solution was washed with water, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to dryness.

Reacrystallization of the residue from ethyl acetate and petroleum ether gave 12.8 g of the crude product (mp 87—96°C). This was again crystallized from the same solvents and 7.84 g of the pure product A was obtained. Concentration and cooling of the mother liquor gave 3.8 g of the mixture of A and *O*,*N*-di-Z(OMe)-L-serine (B). After recrysatllization was repeated twice from ethanol, 1.07 g of B was obtained. From the mother liquor 1.16 g of A was recovered.

Compound A, total yield, 9.0 g. IR spectrum (KBr): 1685 cm^{-1}. TLC (Wakogel B-O): R_f,[18] 0.2.

Found: C, 53.74; H, 5.70; N, 5.18%. Calcd for $C_{12}H_{15}NO_6$: C, 53.53; H, 5.62; N, 5.21%.

Compound B, mp 115—116.5°C (dec). $[\alpha]_D^{17}$ +16.67 (*c* 3, acetic acid). IR spectrum (KBr): 1765, 1650 cm^{-1}. TLC (Wakogel B-O): R_f,[18] 0.6.

Found: C, 58.28; H, 5.41; N, 3.29%. Calcd for $C_{21}H_{23}NO_9$: C, 58.19; H, 5.35; N, 3.23%.

***p*-Methoxybenzyloxycarbonyl-L-valine Dicyclohexylammonium Salt.** Z(OMe)-L-valine was prepared by the same procedure as was used for the Z(OMe)-glycine, *viz.*, an oily residue was obtained from 5.85 g of L-valine (0.05 mol) and 0.1 mol of I. This residue was dissolved in 100 m*l* of ether, and 9.96 m*l* of dicyclohexylamine (0.05 mol) was added at 0—5°C. The white precipitate was filtered and washed with ether. Pure needle crystals were obtained by recrystallization from ethanol and petroleum ether; yield, 9.15 g. IR spectrum (KBr): 1710, 1630 cm^{-1}.

Found: C, 67.26; H, 9.23; N, 6.32%. Calcd for $C_{26}H_{42}N_2O_5$: C, 67.50; H, 9.15: N, 6.05%.

***p*-Methoxybenzyloxycarbonyl-L-leucine Dicyclohexylammonium Salt.** This was synthesized by the same method as was used for the Z(OMe)-L-valine from 6.55 g of L-leucine. Pure dicyclohexylamine salt (21.15 g) was obtained as needle crystals after recrystallization from ethanol and petroleum ether. IR spectrum (KBr): 1710, 1630 cm^{-1}.

Found: C, 68.04; H, 9.07; N, 6.01%. Calcd for $C_{27}H_{44}N_2O_5$: C, 68.03; H, 9.31; N, 5.88%.

***p*-Methoxybenzyloxycarbonyl-L-phenylalanine Dicyclohexylammonium Salt.** This was prepared from 8.25 g of L-phenylalanine by the same method as mentioned above. Pure white needle crystals (19.18 g) were obtained after recrystallization from ethanol and petroleum ether. IR spectrum (KBr): 1700, 1635 cm^{-1}.

Found: C, 70.76; H, 8.35; N, 5.83%. Calcd for $C_{30}H_{42}N_2O_5$: C, 70.55; H, 8.29; N, 5.49%.

***p*-Methoxybenzyloxycarbonyl-L-proline Dicyclohexylammonium Salt.** From 5.75 g of L-proline (0.05 mol), this salt was prepared by the same method as described for Z(OMe)-L-valine dicyclohexylammonium salt. Recrystallization from ethanol and petroleum ether gave 19.02 g of pure minute crystalline plates.

IR spectrum (KBr): 1700, 1635 cm^{-1}.

Found: C, 68.02; H, 8.90; N, 6.20%. Calcd for $C_{26}H_{40}N_2O_5$: C, 67.79; H, 8.76; N, 6.08%.

***p*-Methoxybenzyloxycarbonyl-L-methionine Dicyclohexylammonium Salt.** This *p*-methoxybenzyloxycarbonyl amino acid was prepared from 7.46 g of L-methionine (0.05 mol) and 0.1 mol of I by a procedure similar to the synthesis of L-valine derivative. Its dicyclohexylamine salt was obtained as a white precipitate. Recrystallization of the salt from ethanol and petroleum ether gave pure needle crystals; yield, 21.76 g. IR spectrum (KBr): 1705, 1635 cm^{-1}.

Found: C, 63.14; H, 8.86; N, 5.76%. Calcd for $C_{26}H_{42}N_2O_5S$: C, 63.12; H, 8.56; N, 5.66%.

***α-N-p*-Methoxybenzyloxycarbonyl-*δ-N*-benzyloxycarbonyl-L-ornithine Dicyclohexylammonium Salt.** *α-N-p*-Methoxybenzyloxycarbonyl-*δ-N*-benzyloxycarbonyl-L-ornithine, an oily compound, was prepared from 13.3 g of *δ-N*-benzyloxycarbonyl-L-ornithine[24] (0.05 mol) by the same procedure as for Z(OMe)-glycine. It was dissolved in ether (100 m*l*), and 9.96 m*l* of dicyclohexylamine (0.05 mol) was added at 0—5°C. The white precipitate formed was filtered and washed with ether (14.34 g, mp 129—135°C). This was recrystallized from ethanol and petroleum ether. Yield, 10.2 g of pure product. IR spectrum (KBr): 1695, 1630 cm^{-1}.

Found: C, 66.91; H, 7.91; N, 6.94%. Calcd for $C_{34}H_{49}N_3O_7$: C, 66.75; H, 8.07; N, 6.87%.

The authors wish to express their thanks to the staff of the Research Laboratory of the Chugai Pharmaceutical Co., Ltd., for their elemental analyses.

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[24] B. C. Barrass and D. T. Elmore, *J. Chem. Soc.*, **1957**, 3134.