[Chem. Pharm. Bull. 19(2) 420—423 (1971)]

UDC 547.334.04.07 : 547.466.1.07

Studies on Peptides. XXX.[1] Trichloroethyloxycarbonylhydrazine

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(Received August 7, 1970)

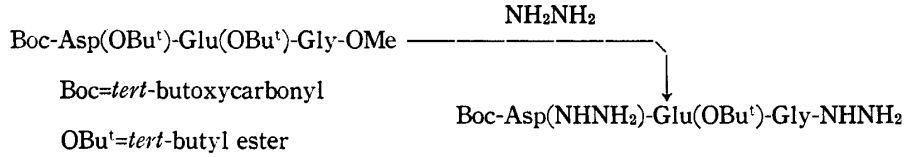
The use of benzyloxycarbonylhydrazine[3] in the peptide synthesis was introduced for the first time by Hofmann, *et al.*[4] It was demonstrated, for example, that phthalylamino acid (or peptide) benzyloxycarbonylhydrazides could serve, after either hydrogenolysis or hydrazinolysis, as the carboxyl component or as the amino component for further elongation of the peptide chain respectively. *tert*-Butoxycarbonylhydrazine[5] and tritylhydrazine[6] were used in an analogous manner.

This approach in the peptide synthesis became especially useful for the preparation of peptide hydrazides containing base-sensitive arginine, since usual conversion of N-protected arginyl (or nitroarginyl) peptide esters to the corresponding hydrazides by hydrazine may cause the cleavage of its guanidino function.[7,8] These peptide hydrazides can only be prepared unequivocally with a protected hydrazine as the C-terminus.

Such demand also arises in the synthesis of peptide hydrazides containing the benzyl or *tert*-butyl ester of aspartic acid.[8,9] This amino acid gives rise to special problem; since the *tert*-butyl ester[10–12] attached to the β-carboxyl group is not only saponified by alkali through the N-succinimide intermediate as like as the benzyl ester[13] but also converted to hydrazide by hydrazine[12] unless extreme care are taken.[10,14] We have also observed this phenomenon, when *tert*-butoxycarbonyl-β-*tert*-butylaspartyl-γ-*tert*-butylglutamylglycine methyl ester was treated with hydrazine. The analytical data of the product so far isolated agreed well with those of the corresponding dihydrazide. Hydrazinolysis of the *tert*-butyl ester of the aspartic acid residue, as well as the C-terminal methyl ester, seems probable, since

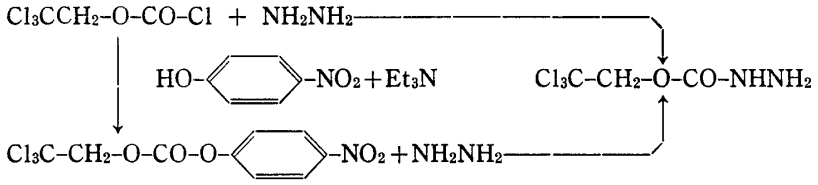
1) Part XXIX: H. Yajima, F. Tamura and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **18**, 2574 (1970).
2) Location: *Sakyo-ku, Kyoto.*
3) N. Rabjohn, *J. Am. Chem. Soc.*, **70**, 1181 (1948) see also H. Boshagen and J. Ullrich, *Chem. Ber.*, **92**, 1478 (1959); E. Wünsch, *ibid.*, **98**, 794 (1965).
4) K. Hofmann, M.Z. Magee and A. Lindenmann, *J. Am. Chem. Soc.*, **72**, 2814 (1950); K. Hofmann, A. Lindenmann, M.Z. Magee and N.H. Khan, *ibid.*, **74**, 470 (1952).
5) L.A. Carpino, *J. Am. Chem. Soc.*, **79**, 98 (1957) see also R.A. Boissonnas, St. Guttmann and P.A. Jaquenoud, *Helv. Chim. Acta*, **43**, 1349 (1960); R. Schwyzer, E.S. Wegman and H. Dietrich, *Chimia*, **14**, 366 (1960).
6) F. Weygand and W. Steglich, *Chem. Ber.*, **92**, 313 (1959).
7) K. Hofmann, W. Haas, M.J. Smithers, R.D. Wells, Y. Wolman, N. Yanaihara and G. Zanetti, *J. Am. Chem. Soc.*, **87**, 620 (1965); N. Yanaihara, T. Hashimoto, C. Yanaihara and N. Sakura, *Chem. Pharm. Bull.* (Tokyo), **18**, 417 (1970).
8) St. Guttmann, *Helv. Chim. Acta*, **44**, 721 (1961).
9) E. Wünsch, G. Wendlberger and J. Jentsch, *Chem. Ber.*, **97**, 3298(1964), E. Wünsch, *ibid.*, **98**, 797(1965); N, Yanaihara, C. Yanaihara, G. Dupuis, J. Beacham, R. Camble and K. Hofmann, *J. Am. Chem. Soc.*, **91**, 2184 (1969).
10) R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel and H. Zuber, *Helv. Chim. Acta*, **46**, 1975 (1963).
11) S. Bajusz, T. Lazar and Z. Paulay, *Acta Chimica Acad. Sci. Hungaricae*, **41**, 329 (1964).
12) F. Chillemi, *Gazz. Chim. Ital.*, **96**, 359 (1966).
13) M.A. Ondetti, A. Deer, J.T. Sheehan, J. Pluscec and O. Kocy, *Biochemistry*, **7**, 4069 (1968) see other reference thereof.
14) R.G. Denkewalter, D.F. Veber, F.W. Holly, R. Hirshmann, R.G. Struchan, W.J. Paleveda, Jr. and R.F. Nutt, *J. Am. Chem. Soc.*, **91**, 502 (1969) see other reference thereof.

the same protecting group at the glutamic acid residue is known to be stable under this treatment.[15]

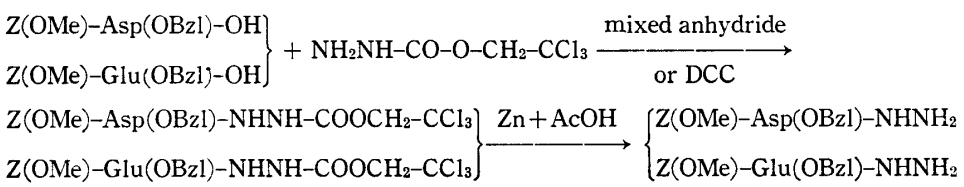


With these considerations, we have now prepared trichloroethyloxycarbonylhydrazine. The protecting group of which was introduced by Woodward, *et al.*[16] and is known to be cleaved more easily than monohalosubstituted ethyloxycarbonyl groups by zinc in methanol or acetic acid.[17,18] These conditions seem not to cleave the benzyloxycarbonyl, *tert*-butoxycarbonyl and *tert*-butyl (or benzyl) ester groups, even if they were previously adopted for the protection of α-amino and other ω-functional groups involved in the synthetic peptide chain.

Trichloroethyloxycarbonyl chloride was prepared as described.[17] It is a distillable and stable reagent. Crystalline trichloroethyloxycarbonylhydrazine was obtained after direct reaction of trichloroethyloxycarbonyl chloride with 80% hydrazine (2 equi-moles) in cold. Elemental analysis agreed well with values predicted by theory and its structure was further spectrometrically confirmed. The same compound was prepared by hydrazinolysis of trichloroethyl *p*-nitrophenyl carbonate. This carbonate, which is crystalline, was prepared in excellent yield by the reaction of trichloroethyloxycarbonyl chloride with *p*-nitrophenol in the presence of triethylamine.



For example, *p*-methoxybenzyloxycarbonyl-β-benzylaspartate and *p*-methoxybenzyloxycarbonyl-γ-benzylglutamate were condensed with trichloroethyloxycarbonylhydrazine by means of the mixed anhydride or dicyclohexylcarbodiimide procedure. Both of the resulting trichloroethyloxycarbonylhydrazides were oil. However, they were treated with zinc powder in acetic acid at room temperature for 1 hr, *p*-methoxybenzyloxycarbonyl-β-benzylaspartate hydrazide and *p*-methoxybenzyloxycarbonyl-γ-benzylglutamate hydrazide were obtained in a crystalline form. Elemental analyses were in good agreement with values



Z(OMe)=*p*-methoxybenzyloxycarbonyl, OBzl=benzyl ester

15) H. Kappeler and R. Schwyzer, *Helv. Chim. Acta*, **44**, 1136 (1961); R. Schwyzer and H. Kappeler, *ibid.*, **44**, 1991 (1961).
16) R.B. Woodward, K. Heusler, J. Gosteli, W. Oppolzer, R. Ramage, S. Ranganathan and H. Vorbruggen, *J. Am. Chem. Soc.*, **88**, 852 (1966).
17) T.B. Windholz and D.B.R. Johnston, *Tetrahedron Letters*, **1967**, 2555.
18) J. Grimshaw, *J. Chem. Soc.*, **1965**, 7136.

predicted by theory. These two hydrazides exhibited sharp blue spots on thin layer chromatography on silica gel by the hydrazine test.[19]

Application of trichloroethyloxycarbonylhydrazine, including these above stated aspartic acid and glutamic acid derivatives, in the peptide synthesis will be reported in furture.

Experimental

Trichloroethyloxycarbonyl Chloride——The title compound was prepared according to Windholz and Johnston.[17] bp 105—106°/90 mmHg (lit.[17] bp 171—172°/760 mmHg, 75—76°/60 mmHg).

Trichloroethyl *p*-Nitrophenyl Carbonate——Triethylamine (5.95 ml) was added dropwise to a well stirred solution of trichloroethyloxycarbonyl chloride (9.0 g) and *p*-nitrophenol (5.91 g) in ether (150 ml) at room temperature for 1 hr and the mixture was stirred for an additional 1 hr. After addition of H_2O, the organic phase was washed with 0.5N HCl and a saturated solution of NaCl, dried over Na_2SO_4 and then evaporated. Trituration of the residue with petroleum ether gave solid, which was recrystallized from EtOH and petroleum ether; yield 12.17 g (91%), mp 58°. *Anal.* Calcd. for $C_9H_6O_5NCl_3$: C, 34.37; H, 1.92; N, 4.45. Found: C, 34.53; H, 1.94; N, 4.18.

Trichloroethyloxycarbonylhydrazine——a) Direct Preparation: To a mixture of 80% hydrazine hydrate (12 ml, 0.2 mole) in ether (100 ml), trichloroethyloxycarbonyl chloride (21 g, 0.1 mole) in ether (200 ml) was added with vigorous stirring during a period of 2 hr under cooling with ice–NaCl. Stirring was continued for 1 hr. The organic phase was extracted with 1N HCl (100 ml×3), which in turn was basified with 1N Na_2CO_3. The resulting precipitate was extracted with ether, which was dried over Na_2SO_4 and then evaporated. The residue was crystallized by trituration with petroleum ether; yield 14.0 g (68%), mp 42—44°. NMR: τ 5.23 (–CH_2–), 3.45 (–NH–), 6.12 (–NH_2). Mass Spectra: $M^+=m/e=206$. IR: 1740 cm^{-1} (–CO–), 3400 cm^{-1} (NH_2). *Anal.* Calcd. for $C_3H_5O_2N_2Cl_3$: C, 17.37; H, 2.43; N, 13.50. Found: C, 17.48; H, 2.51; N, 13.72.

b) Through *p*-Nitrophenyl Carbonate: A mixture of trichloroethyl *p*-nitrophenyl carbonate (6.29 g) and 80% hydrazine hydrate (2.43 ml) in methanol (120 ml) was stirred at room temperature for 1 hr. The solvent was evaporated and the residue was dissolved in ether, which was washed with 1N Na_2CO_3 and a solution of NaCl, dried over Na_2SO_4 and then evaporated. The residue turned to solid by trituration with petroleum ether under cooling with ice; yield 3.70 g (89.4%), mp 42—44°. *Anal.* Found: C, 17.66; H, 2.50; N, 13.70.

***p*-Methoxybenzyloxycarbonyl-*β*-benzylaspartate**——*p*-Methoxybenzyl azidoformate was prepared according to our newly devised procedure.[20] This azidoformate (20.70 g) in dioxane (200 ml) was added to a solution of *β*-benzylaspartate (22.32 g) and triethylamine (21 ml) in 25% aqueous pyridine (250 ml) and the mixture was stirred at room temperature for 8 hr. The solvent was evaporated and the residue was dissolved in H_2O, which after washing with ether, was acidified with ice-cold 10% citric acid and the resulting precipitate was extracted with AcOEt. The organic phase was washed with a saturated solution of NaCl, dried over Na_2SO_4 and then evaporated. The residue was crystallized with ether and recrystallized from the same solvent; yield 31.41 g (81%). mp 90—92°. *Anal.* Calcd. for $C_{20}H_{21}O_7N$: C, 62.0; H, 5.5; N, 3.6. Found: C, 62.1; H, 5.5; N, 3.6. Analytical data of the title compound assigned by Weygand and Nintz[21] do not agree with those of the desired benzyl ester. They used magnesium oxide instead of triethylamine. Their result may due to the saponification of the benzyl ester occured during the reaction.

***p*-Methoxybenzyloxycarbonyl-*β*-benzylaspartate Trichloroethyloxycarbonylhydrazide**——A mixed anhydride was prepared in the usual manner from *p*-methoxybenzyloxycarbonyl-*β*-benzylaspartate (1.94 g) with triethylamine (0.7 ml) and ethyl chloroformate (0.48 ml) in dry tetrahydrofuran (THF) (20 ml). To this solution, trichloroethyloxycarbonylhydrazine (1.04 g) in THF (5 ml) was added and the mixture was stirred in an ice–bath for 2 hr, when the solvent was evaporated. The residue was dissolved in AcOEt, which after washing with 10% citric acid, 1N $NaHCO_3$ and a saturated solution of NaCl, was dried over Na_2SO_4 and then evaporated to give an oily product; yield 2.70 g (93%). *Anal.* Calcd. for $C_{23}H_{24}O_8N_3Cl_3$: C, 47.89; H, 4.19; N, 7.28. Found: C, 48.07; H, 4.25; N, 7.57. The same compound can be prepared by the DCC procedure; yield 90%.

***p*-Methoxybenzyloxycarbonyl-*β*-benzylaspartate Hydrazide**——The above oily trichloroethyloxycarbonyl derivative (1.10 g) in AcOH (10 ml) was treated with zinc powder (0.7 g) at room temperature for 1 hr. After filtration, the filtrate was made alkaline with 1N Na_2CO_3 and the resulting product was extracted with AcOEt, which after filtration, was washed with a saturated solution of NaCl, dried over Na_2SO_4 and then

19) K. Hofmann, R. Schmiechen, R.D. Wells, Y. Wolman and N. Yanaihara, *J. Am. Chem. Soc.*, **87**, 611 (1965).
20) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **17**, 1962 (1969); H. Yajima, H. Kawatani and Y. Kiso, *ibid.*, **18**, 850 (1970).
21) F. Weygand and E. Nintz, *Z. Naturforschg.*, **20b**, 429 (1965).

evaporated. The residue was crystallized by trituration with ether and petroleum ether; yield 0.64 g (80%), mp 110—115°. *Anal.* Calcd. for $C_{20}H_{23}O_6N_3$: C, 59.84; H, 5.78; N, 10.47. Found: C, 59.70; H, 5.84, N, 10.18.

***p*-Methoxybenzyloxycarbonyl-γ-benzylglutamate**——The title compound was prepared by Weygand and Hunger[22] and Sakakibara, *et al.*[23] but their procedures were different from our present procedure. *p*-Methoxybenzyl azidoformate (62.2 g) in dioxane (600 ml) was added to a mixture of γ-benzylglutamate (71.1 g) and MgO (28.2 g) in H_2O (600 ml). After stirring at room temperature for 24 hr., the solution was filtered and the filtrate was condensed *in vacuo*. The residue was dissolved in H_2O, which after washing with ether, was acidified with 10% citric acid. The resulting precipitate was extracted with AcOEt, which was washed with a saturated solution of NaCl, dried over Na_2SO_4 and then evaporated. Addition of petroleum ether to the residue gave solid, which was recrystallized from AcOEt and petroleum ether; yield; 110 g (92%), mp 75—76°, (lit.[23] mp 87—88.5°). *Anal.* Calcd. for $C_{21}H_{23}O_7N$: C, 62.8; H, 5.8; N, 3.5. Found: C, 62.6; H, 6.1; N, 3.7.

***p*-Methoxybenzyloxycarbonyl-γ-benzylglutamate Trichloroethyloxycarbonylhydrazide**——Isobutyl chloroformate (0.13 ml) was added to an ice-cold solution of *p*-methoxybenzyloxycarbonyl-γ-benzylglutamate (0.40 g) and triethylamine (0.14 ml) in dry THF (5 ml) and the mixture was stirred for 30 min. To this solution, trichloroethyloxycarbonylhydrazine (0.21 g) in THF (3 ml) was added. The mixture was stirred in an ice-bath for 2 hr, when the solvent was evaporated. The residue was dissolved in AcOEt, which after washing with 10% citric acid, $NaHCO_3$ and a solution of NaCl, was dried over Na_2SO_4 and then evaporated to give an oily product; yield 0.50 g (85%). This trichloroethyloxycarbonylhydrazine derivative can also be obtained by the DCC procedure; yield; 85%.

***p*-Methoxybenzyloxycarbonyl-γ-benzylglutamate Hydrazide**——The oily product, *p*-methoxybenzyloxycarbonyl-γ-benzylglutamate trichloroethyloxycarbonylhydrazine, (0.50 g) was dissolved in AcOH (10 ml) and zinc powder (0.6 g) was added. After stirring at room temperature for 1.5 hr, the solution was filtered and the filtrate was diluted with H_2O. The aqueous solution was washed with AcOEt and then made alkaline with 1N Na_2CO_3. The resulting precipitate was extracted with AcOEt, which after filtration and subsequent washing with a saturated solution of NaCl, was dried over Na_2SO_4 and then evaporated. The residue was solidified with ether; yield; 0.29 g (85%), mp 85—90°. *Anal.* Calcd. for $C_{21}H_{25}O_6N_3$: C, 60.71; H, 6.07; N, 10.11. Found: C, 60.77; H, 6.32; N, 9.82.

Treatment of Boc-Asp(OBu^t)-Glu(OBu^t)-Gly-OMe with Hydrazine——Boc-Asp(OBu^t)-Glu(OBu^t)-Gly-OH[24] (1.14 g) was methylated by diazomethane. The oily methyl ester thus obtained (1.08 g) was dissolved in MeOH (7 ml) and 80% hydrazine hydrate (0.38 ml) was added. The solution was kept on standing at room temperature for 3 days and ether was added to form crystalline solid, which was collected by filtration; yield 0.30 g (30%), mp 197—198°. *Anal.* Calcd. for $C_{20}H_{37}O_8N_7$: C, 47.7; H, 7.4; N, 19.5. Found: C, 47.9; H, 7.7; N, 19.5.

22) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 7 (1962).
23) S. Sakakibara, I. Honda, M. Naruse and M. Kanaoka, *Exp.*, **25**, 576 (1969).
24) H. Yajima, Y. Okada, Y. Kinomura, N. Mizokami and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **17**, 1237 (1969).