Communications to the Editor

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PROPERTIES OF N^{α},N^{ca}-DI-TERT-BUTYLOXYCARBONYL-ω-CARBAMOYL-α-AMINO ACIDS AND DIRECT SYNTHESIS OF PROTECTED HOMOGLUTAMIC ACID DERIVATIVES

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The protected carboxamide function of N^{α},N^{ca}-di-tert-butyloxycarbonyl-ω-carbamoyl-α-amino acids worked well with nucleophilic reagents. Applying this novel reactivity, we developed an efficient synthetic route to N^{α}-tert-butyloxycarbonylhomoglutamic acid and its derivatives, including N^{α}-tert-butyloxycarbonylhomoglutamic acid δ-benzyl ester, from N^{α},N^{ca}-di-tert-butyloxycarbonylhomoglutamine.

KEYWORDS ——— protected homoglutamic acid synthesis; N^{ca}-tert-butyloxycarbonylated carboxamide; hydrolysis; selective deprotection; N^{α}-tert-butyloxycarbonylhomoglutamic acid δ-benzyl ester; N^{α}-tert-butyloxycarbonylhomoglutamic acid α-tert-butyl ester; N^{α}-tert-butyloxycarbonylhomoglutamine; optical purity

Recently we reported the synthesis of L-asparagine and L-glutamine derivatives carrying the carboxamide nitrogen (N^{ca}) acylated by urethane type protecting groups and the possible application of this method to peptide synthesis.[1] The novel amide protecting groups have similar resistibility and lability to the same groups on amine under the representative cleavage conditions.[1] However, in the synthesis work, N^{α},N^{ca}-di-tert-butyloxycarbonyl-ω-carbamoyl-α-amino acids have been prepared from the corresponding C^{α}-4-nitrobenzyl or C^{α}-2,2,2-trichloroethyl esters through catalytic hydrogenation or zinc treatment in acetic acid, but not from the methyl esters through saponification due to instability of the protected amino acids.

Now we have examined the stability of N^{ca}-protected ω-carbamoyl-α-amino acids and their decomposed products. Based upon the disclosed chemical characteristics of N^{ca}-tert-butyloxycarbonylated carboxamide function, an efficient and direct synthesis of protected homoglutamic acid (Hgu; α-aminoadipic acid) derivatives was explored.

First, the stability of N^{α},N^{ca}-di-tert-butyloxycarbonylasparagine [Boc-Asn(Boc)-OH] and N^{α},N^{ca}-di-tert-butyloxycarbonylglutamine [Boc-Gln(Boc)-OH] was examined qualitatively under various conditions commonly used in peptide synthesis.[2] The decrease of the starting materials was determined by reversed phase high performance liquid chromatography (RP-HPLC) as well as TLC at regular time intervals under isocratic or linear gradient conditions of the eluent systems. No considerable degradation was observed in acetic acid, triethylamine in methylene chloride, or sodium bicarbonate solution, nor for one month in methanol in which Z-pGlu-peptide[3] is reported to be unstable. Catalytic hydro-

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genation did not affect these N^{α},N^{ca}-di-tert-butyloxycarbonyl compounds. The protecting group of N^{ca}-Boc was deblocked simultaneously with N^{α}-Boc in trifluoroacetic acid or in 25% hydrogen bromide in acetic acid to give asparagine and glutamine. Interestingly even in 5% trifluoroacetic acid in methylene chloride, the peaks of the starting materials, Boc-Asn(Boc)-OH or Boc-Gln(Boc)-OH, disappeared in 1 h, and new peaks appeared in the HPLC chromatograms of both reaction mixtures. These peaks were found to have the same retention times as those of Boc-Asn-OH and Boc-Gln-OH, respectively. This demonstrates the predominant cleavage of N^{ca}-Boc and the survival of N^{α}-Boc under the mild acidic condition. It was also of interest that Boc-Asn(Boc)-OH and Boc-Gln(Boc)-OH easily reacted with 1 N sodium hydroxide solution, which has no effect on N^{α}-Boc, producing new peaks on HPLC chromatograms. These peaks were assigned to Boc-Asp-OH and Boc-Glu-OH, respectively, by comparison with authentic samples. Converting N^{ca}-unprotected asparagine and glutamine derivatives to aspartic acid and glutamic acid derivatives by the use of 1 N sodium hydroxide solution is well known but the reaction is somewhat slower. Accordingly the hydrolysis products were considered to be derived by direct substitution of Boc-Asn(Boc)-OH and Boc-Gln(Boc)-OH and not from Boc-Asn-OH and Boc-Gln-OH which may be produced by deprotection of N^{ca}-Boc. This was further confirmed by isolation of tert-butyl carbamate (Boc-NH_2) from the reaction mixture. These results clearly indicate that the N^{ca}-protected amide carbonyl functions of asparagine and glutamine have acquired increased reactivity with nucleophilic reagents, resulting in the decreased stability of N^{α},N^{ca}-di-tert-butyloxycarbonylamino acids in alkaline solution.

Thus, the chemical property of the carboxamide function with N^{ca}-Boc was used to produce Boc-Hgu-OH and its derivatives directly in optically pure forms from Boc-Hgn(Boc)-OH [L-form : mp 72-74°C, $[\alpha]_D^{17}$ -1.5° (c=1, MeOH)]. The latter was prepared from N^{α},N^{ε}-di-tert-butyloxycarbonyllysine 2,2,2-trichloroethyl ester [Boc-Lys(Boc)-OTce, L-form : mp 100-101°C, $[\alpha]_D^{23}$ -19.1° (c=1, AcOEt)] by ruthenium tetroxide oxidation[4] followed by treatment with zinc in acetic acid. This is the method reported previously for the corresponding asparagine and glutamine derivatives.[1]

The synthetic route to Boc-Hgu-OH and its derivatives is shown in the Chart. The higher acid lability of N^{ca}-Boc made it possible to convert Boc-Hgn(Boc)-OH directly to Boc-Hgn-OH in good yield under the strictly controlled condition of 5% trifluoroacetic acid in methylene chloride at 20°C for 30 min, although the deblock of N^{ca}-Boc was accompanied by removal of N^{α}-Boc to some extent to yield homoglutamine. Hydrolysis of Boc-Hgn(Boc)-OH with 1 N sodium hydroxide (2.2 eq mol) in methanol at 20°C for 1 h gave Boc-Hgu-OH almost quantitatively. Simultaneous hydrolysis of the α-ester and the protected carboxamide of Boc-Hgn(Boc)-OTce [L-form : mp 56-58°C, $[\alpha]_D^{23}$ -16.8° (c=1, AcOEt)] or Boc-Hgn(Boc)-OMe[4] in alkaline solution proved to be a simple procedure for producing Boc-Hgu-OH. Hydrazinolysis gave Boc-Hgu(N_2H_3)-OH. By a similar reaction with anhydrous sodium benzylate in benzyl alcohol, Boc-Hgn(Boc)-OH yielded directly Boc-Hgu(OBzl)-OH[5] in 70-80% yield. This procedure is indispensable for the synthesis of Hgu-containing peptides. Boc-Hgn(Boc)-OBu^t [4] was important not only for homoglutamine preparation by unselective cleavage with neat trifluoroacetic acid or for homoglutamic acid with 6 N HCl hydrolysis,

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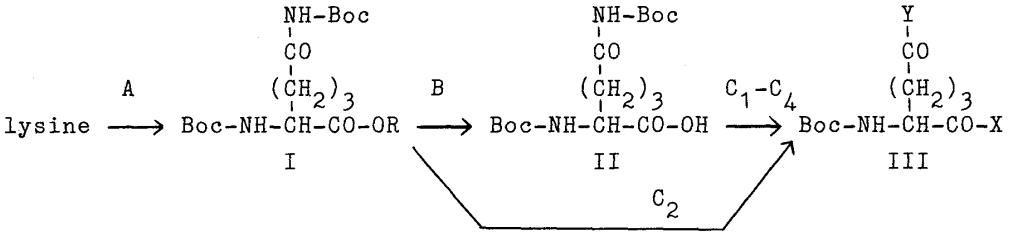
Chart. Synthetic Route to Protected Homoglutamic Acid Derivatives



-X, -Y = functional group (see Table); -OR = -OTce, -OMe or -OBu^t; Procedure A = 1) Boc-ON, 2) R-OH/DCC/DMAP or isobutylene, 3) RuO_4; B = Zn/AcOH when -OR is -OTce; C_1 = 5% TFA/CH_2Cl_2 at 20°C for 30 min; C_2 = 1N NaOH in MeOH at 20°C for 1 h; C_3 = $BzlO^-Na^+$ in BzlOH at 25°C for 3 h; C_4 = N_2H_4 (10 eq mol) in MeOH at 20°C for 16 h.

Table. Synthesis and Characterization of Boc-Hgu(Y)-X

Product III							
X	Y	Optical form	Substrate I(OR) or II	Procedure	Yield (%)	mp (°C)	$[\alpha]_D^{17}$ (c=1,MeOH)
OH	NH_2	L	II	C_1	63	162-163	-4.9°
		D	II	C_1	67	161-162	+4.5°
OH	OH	L	II	C_2	91	126-127	-7.8°
		L	I(OMe)	C_2	95		
		D	II	C_2	92	126-127	+7.6°
OH·DCHA	OBzl	L	II	C_3	72	122-124	+9.9°
		D	II	C_3	80	120-122	-10.0°
OBu^t	OH	L	I(OBu^t)	C_2	87	75-77	-24.8°
		D	I(OBu^t)	C_2	88	75-77	+23.9°
OH	N_2H_3	L	II	C_4	79	59-64	-1.4°
OBu^t	N_2H_3	L	I(OBu^t)	C_4	83	oil	-13.9°

but also for direct preparation of Boc-Hgu-OBu^t [6] by selective hydrolysis with sodium hydroxide solution to form the free δ-carboxylic acid. This acid should be a good derivative for synthesizing β-lactam antibiotics or their precursor peptides.

The protected homoglutamic acid derivatives synthesized in this study gave

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reasonable analytical data in elemental analysis. Also, FAB-MAS gave M + 1, M + 23, M - 55 (isobutylene) and other peaks, which were 14 ($-CH_2-$) or 28 ($-CH_2-CH_2-$) mass higher than the corresponding glutamic acid or aspartic acid derivatives. The optical purity of the products, originating from L- or D-lysine, was certified to be conserved by the method reported previously.[1)]

Finally, ammonolysis of Boc-Hgn(Boc)-OH, being similar to the reactions described above, proceeded slowly with ammonia saturated in methanol at room temperature for 7 days. The yield of Boc-Hgn-OH was about 30%. This suggests that neglegible aminolysis by an amino component could take place at the ω-carbonyl function of N^{α},N^{ca}-di-tert-butyloxycarbonylamino acid during coupling reaction for α-peptide bond formation, as reported[2)] in the synthesis of a Hgn^6-substance P analog.

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