Communications to the Editor

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URETHANE TYPE PROTECTING GROUPS FOR CARBOXAMIDE IN PEPTIDE CHEMISTRY

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Novel L-asparagine and L-glutamine derivatives with the carboxamide nitrogen(N^{ca}) acylated by urethane type protecting groups, tert-butyloxycarbonyl(Boc) and 4-nitrobenzyloxycarbonyl [$Z(NO_2)$], were synthesized through ruthenium tetroxide oxidative transformation of N^{α},N^{ω}-diacylated L-2,4-diaminobutyric acid and L-ornithine. N^{ca}-Boc and N^{ca}-$Z(NO_2)$ groups can be deprotected by trifluoroacetic acid and catalytic hydrogenation respectively. N^{α},N^{ca}-DiBoc-Asn-OH is applied to peptide synthesis by dicyclohexylcarbodiimide or mixed anhydride method without nitrile formation.

KEYWORDS —— carboxamide nitrogen (N^{ca}) protection; N^{ca}-tert-butyloxycarbonyl; N^{ca}-4-nitrobenzyloxycarbonyl; trifluoroacetic acid deprotection; catalytic hydrogenation; ruthenium tetroxide oxidation; peptide synthesis; N^{α},N^{ca}-di-tert-butyloxycarbonyl-L-asparagine; dehydration inhibition; HPLC

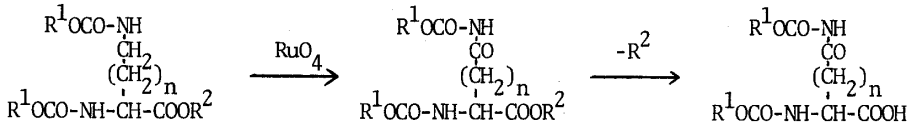
Dehydration of carboxamide group to nitrile is well known to take place during the activation of N^{α}-acylated asparagine or glutamine in peptide synthesis. To avoid or diminish the side reaction several carboxamide protecting groups have been developed. The introduction of protecting group into the carboxamide function is restricted to a substitution by benzhydrol derivatives.[1] An alternative way involves an amidation of ω-carboxylic acid of aspartic acid or glutamic acid with primary or secondary benzylamines to give benzyl type protecting groups.[2] These protecting groups are deblocked exclusively by acids.

Now we report here synthesis of novel L-asparagine and L-glutamine derivatives bearing urethane type protecting groups, tert-butyloxycarbonyl (Boc) and 4-nitrobenzyloxycarbonyl [$Z(NO_2)$], on the carboxamide nitrogen (N^{ca}), and the possible application to peptide synthesis. Urethane type protecting groups are the most important and widely used for amine protection because of the great variety of cleavage conditions dependent on the ester moiety. They are sometimes used as well for protection of side chain nitrogens of amino acids, i.e., N^{im} of histidine, N^{G} of arginine and N^{in} of tryptophan,[3] but no application to N^{ca} of asparagine or glutamine has been reported. The chemical fundamentals of the synthesis are not the direct insertion of the protecting groups into the amide function, nor the amidation, but a conversion reaction of L-α,ω-diamino acids, acylated on both amines by urethane forming, into the corresponding L-ω-amido-α-amino acids through ruthenium tetroxide (RuO_4) oxidation.[4]

The starting materials, N^{α},N^{ω}-dialkyloxycarbonyldiamino acid esters (Ia-d in the Chart), were prepared from L-2,4-diaminobutyric acid or L-ornithine with tert-butyl S-4,6-dimethylpyrimid-2-ylthiocarbonate[5] (Kokusan) or $Z(NO_2)$-Cl (Aldrich), followed by esterification with 4-nitrobenzyl alcohol in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine[6] or with isobutylene.

The key step RuO_4 oxidation transforms I into the skeleton changed amino acid (II), where the methylene adjacent to ω-amine of the α,ω-diamino acid is susceptible to the oxidation to generate carboxamide function with N^{ca}-urethane type protecting group; on the other hand, α-carbon is kept intact by the chemoselective character of the reagent. The reaction was effected by employing a

Chart. Synthesis of L-Asparagine and L-Glutamine Derivatives Bearing N^{ca}-Boc and N^{ca}-$Z(NO_2)$



Ia-d IIa-d IIIa-d

	n	R^1	R^2	Yield (%) II	III
a	1	Bu^t	Nb	58 (84*)	92
b	1	Nb	Bu^t	37	90
c	2	Bu^t	Nb	54 (73*)	88
d	2	Nb	Bu^t	44	79

Bu^t = $-C(CH_3)_3$ Nb = $-CH_2-C_6H_4-NO_2$ (*) = in ref. 4

two-phase (AcOEt-H_2O)[4] catalytic procedure exemplified for N^{α},N^{ca}-diBoc-Gln-ONb (IIc) as follows. To an aqueous solution (50 ml) of $NaIO_4$ (4.28 g, 20 mmol) was added $RuO_2 \cdot xH_2O$ (100 mg, Aldrich), and the resulting RuO_4 solution colored to deep yellow was combined with a solution of N^{α},N^{γ}-diBoc-Orn-ONb (Ic) (2.34 g, 5 mmol) in AcOEt (50 ml). The mixture was stirred at room temperature for 15 h and the organic phase separated was treated with isopropanol (0.5 ml) to remove the catalyst as RuO_2 precipitate. After filtration the solvent was evaporated to give crude IIc which was passed through a column (3 x 90 cm) of Sephadex LH 20 using isopropanol as eluent and solidified with pet. ether (1.30 g, 54%). The other compounds, IIa, IIb and IId, were also isolated in a similar manner and further purified by silica gel chromatography if necessary. Characterization of II is shown in the Table. It takes a longer time for the complete conversion of L-2,4-diaminobutyric acid derivatives to L-asparagine than for L-ornithine to L-glutamine, and compounds with $Z(NO_2)$ take longer than Boc. Lower yields of IIb and IId might be attributable to partial decomposition of benzene ring(s), consuming about three times more $NaIO_4$ than described above.

The resistibility of the carboxamide protecting groups of II under the cleavage conditions of co-existing esters permitted preparation of the useful compound III in peptide synthesis; that is, N^{α},N^{ca}-diBoc-Asn-OH (IIIa) and N^{α},N^{ca}-diBoc-Gln-OH (IIIc) were obtained almost quantitatively by catalytic hydrogenation (H_2/Pd) of the corresponding 4-nitrobenzyl esters (IIa and IIc) and N^{α},N^{ca}-di$Z(NO_2)$-Asn-OH (IIIb) and N^{α},N^{ca}-di$Z(NO_2)$-Gln-OH (IIId) by trifluoroacetic acid (TFA) treatment of the corresponding tert-butyl esters (IIb and IId) respectively.

Deprotection procedure of N^{α}-Boc or N^{α}-$Z(NO_2)$ of III by TFA or H_2/Pd gives rise to simultaneous removal of N^{ca}-Boc and N^{ca}-$Z(NO_2)$. In other words, the protecting groups on carboxamide can be deblocked by the same mild reagents as those for the groups on amine. Under the mild conditions III yielded L-asparagine or L-glutamine, and by 5.7N HCl hydrolysis L-aspartic acid or L-glutamic acid. These amino acids were confirmed by comparative examination with authentic samples by means of TLC, amino acid analysis and optical rotation. The optical purity was analyzed by reversed phase high performance liquid chromatography (RP-HPLC) using the fluorescent chiral adducts of asparagine, glutamine, aspartic acid and glutamic acid with o-phthaldialdehyde and N-acetyl-L-cysteine. By this complete separation system of the four pairs of enantiomers, no racemization was proved clearly during the whole procedure.[7]

Table. Characterization of I, II and III

Compound	mp (°C)	$[\alpha]_D$ *	Rf^I **	Rf^{II} ***	Formula	Elemental analysis (%) Found (Calcd) C	H	N
Ia	99-101	-11.1°	0.35	0.68	$C_{21}H_{31}N_3O_8$	55.96 (55.62	7.18 6.89	9.07 9.27)
Ib	oil	-15.6°	0.12	0.48	$C_{24}H_{28}N_4O_{10}$	54.28 (54.13	5.32 5.30	10.40 10.52)
Ic	136-137	-6.6°	0.38	0.66	$C_{22}H_{33}N_3O_8$	56.44 (56.52	7.07 7.11	8.95 8.99)
Id	99-100	+1.5°	0.12	0.46	$C_{25}H_{30}N_4O_{10}$	54.88 (54.94	5.57 5.63	9.99 10.25)
IIa	121-123	+5.4°	0.35	0.64	$C_{21}H_{29}N_3O_9$	53.86 (53.96	6.27 6.25	8.75 8.99)
IIb	80-82	-3.2°	0.09	0.39	$C_{24}H_{26}N_4O_{11}$	53.14 (52.75	5.00 4.80	9.89 10.25)
IIc	105-106	-6.6°	0.30	0.58	$C_{22}H_{31}N_3O_9$	54.45 (54.88	6.35 6.49	8.50 8.73)
IId	89-92	-5.7°	0.09	0.38	$C_{25}H_{28}N_4O_{11}$	53.22 (53.57	5.15 5.04	9.91 10.00)
IIIa	90-93	+19.5°	0.61	0.73	$C_{14}H_{24}N_2O_7$	50.46 (50.60	7.37 7.28	8.04 8.43)
IIIb	176-178	+10.3°	0.35	0.72	$C_{20}H_{18}N_4O_{11}$	48.91 (48.99	3.63 3.70	11.17 11.43)
IIIc	95-98	+9.5°	0.59	0.74	$C_{15}H_{26}N_2O_7$	52.24 (52.01	7.66 7.47	8.12 8.09)
IIId	139-142	+0.4°	0.40	0.72	$C_{21}H_{20}N_4O_{11}$	50.45 (50.01	4.10 4.00	11.00 11.11)

* $[\alpha]_D^{18}$ (c=1.0, AcOEt) for I and II; $[\alpha]_D^{15}$ (c=1.0, dioxane) for III.

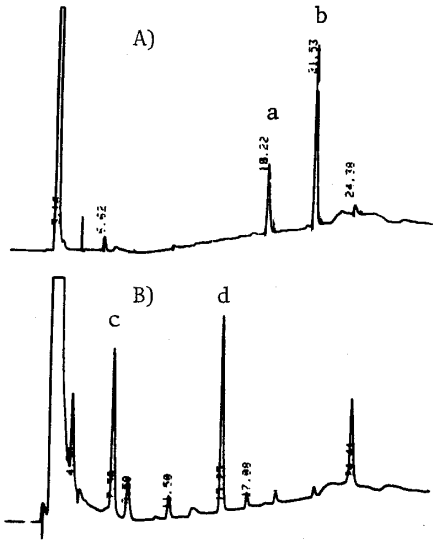
** Rf^I = AcOEt:n-hexane (35:65) for I and II; Rf^I = AcOH:pyridine: benzene (3:27:70) for III.

*** Rf^{II} = isopropanol:acetone:n-hexane (10:15:75) for I and II; Rf^{II} = n-BuOH:AcOH:H_2O (4:1:5, upper phase) for III.

A model protected dipeptide was synthesized by condensation of N^{α},N^{ca}-diBoc-Asn-OH (IIIa) and H-Ala-OMe with DCC or mixed anhydride (MA) using isobutyl chloroformate in tetrahydrofuran (THF)-dimethylformamide (DMF) (9 : 1). The isolated yield of N^{α},N^{ca}-diBoc-Asn-Ala-OMe [oil, $[\alpha]_D^{15}$ -0.7° (c=1.0, AcOEt), Anal. Calcd for $C_{18}H_{31}N_3O_8$: C, 51.79; H, 7.48; N, 10.07. Found ; C, 51.73; H, 7.55; N, 9.73.] was 79% or 91% (DCC or MA method). No nitrile formation was proved by RP-HPLC examination of the DCC reaction mixture, even in the absence of 1-hydroxybenzotriazole (HOBt). In comparative work on the reaction mixture of Boc-Asn-OH and H-Ala-OMe with DCC in THF-DMF (2 : 1), considerable amounts of dehydrated product were observed as shown in the Figure, where the peaks of Boc-Asn-Ala-OMe [mp 174-175°C, $[\alpha]_D^{15}$ -16.4° (c=1.0, DMF), Anal. Calcd for $C_{13}H_{23}N_3O_6$: C, 49.20; H, 7.31; N, 13.24. Found : C, 49.38; H, 7.38; N, 13.03.] and Boc-Ala(CN)-Ala-OMe [mp 127-128°C, $[\alpha]_D^{15}$ -15.1° (c=1.0, AcOEt), Anal. Calcd for $C_{13}H_{21}N_3O_5$: C, 52.17; H, 7.07; N, 14.04. Found : C, 52.40; H, 7.35; N, 14.03.] were identical with those of the authentic samples, which were prepared by the reaction of Boc-Asn-OH or Boc-Ala(CN)-OH[8] with H-Ala-OMe by DCC-HOBt.

It is worthy of remark that L-asparagine and L-glutamine derivatives with N^{ca}-Boc and N^{ca}-Z(NO_2) can be synthesized by the application of RuO_4 oxidative transformation of L-α,ω-diamino acids and

Fig. RP-HPLC Analysis of Coupling Reaction Mixture



A) Boc-Asn(Boc)-OH (a) + H-Ala-OMe
↓ DCC
Boc-Asn(Boc)-Ala-OMe (b)

B) Boc-Asn-OH + H-Ala-OMe
↓ DCC
Boc-Asn-Ala-OMe (c)
+ Boc-Ala(CN)-Ala-OMe (d)

Column : Chemcosorb 7ODS-L (4 x 300 mm); flow rate : 1 ml/min; detection : 210 nm; eluent system : linear gradient from 22% to 43% CH_3CN (15 min) in 20 mM phosphate buffer (pH 3.0).

that Boc and $Z(NO_2)$ groups on carboxamide nitrogen have been found to have similar resistibility and lability to these groups on amine under the representative cleavage conditions. Furthermore, N^{ca}-Boc prevents completely the nitrile formation during the activation of the α-carboxylic group with DCC or MA method. These results suggest the general applicability of urethane type protecting groups to carboxamide function with a variety of mild and selective cleavage conditions in peptide chemistry.

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