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Benzyl and *p*-Nitrobenzyl Monoesters of *N*-t-Butyloxycarbonyl-L-Glutamic Acid and *N*-t-Butyloxycarbonyl-L-Aspartic Acid

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Summary: Different benzyl and *p*-nitrobenzyl monoesters of *N*-Boc-L-glutamic and -L-aspartic acid have been synthesized, partly by modification of already described procedures. α-*p*-nitrobenzyl esters of *N*-Boc-protected aspartic and glutamic acid are suitable intermediates for the preparation of ω-protected *N*-Boc-asparagine and *N*-Boc-glutamine. The *p*-nitrobenzyl group seems favourable for protecting the side-chain carboxylic acid groups of aspartic and glutamic acid under the conditions of solid-phase synthesis.

Zusammenfassung: *Benzyl- und p-Nitrobenzyl-monoester der N-t-Butyloxycarbonyl-L-glutamin- und -asparaginsäure.* Verschiedene Benzyl- und *p*-Nitrobenzyl-monoester der *N*-Boc-L-glutamin- und -asparaginsäure wurden — z.T. durch Modifizierung bereits beschriebener Methoden — synthetisiert. α-*p*-Nitrobenzylester der *N*-Boc-geschützten Asparagin- und Glutaminsäure sind geeignete Zwischenprodukte für die Darstellung von ω-geschütztem *N*-Boc-Asparagin und *N*-Boc-Glutamin. Die *p*-Nitrobenzyl-Gruppe ist unter den Bedingungen der MERRIFIELD-Synthese eine günstige Schutzgruppe für die Seitenketten-Carboxylgruppen der Asparagin- und Glutaminsäure.

With the increasing application of the technique of stepwise chain elongation to the synthesis of biologically important peptides and protected peptide fragments thereof[1–5], the rational synthesis of suitable side-chain protected *N*-Boc-amino acid derivatives has acquired growing importance. Thus if the carbodiimide coupling method[6] is used for aspartic or glutamic acid, one carboxyl group must be protected by an ester linkage that is stable enough to allow the selective acidolytic removal of the Boc group.

In planning the synthesis of the coat protein of tobacco mosaic virus *(vulgare)*, which consists of 158 amino acid residues, the presence of 10 asparagine, 10 glutamine, 8 aspartic acid and 6 glutamic acid residues motivated an investigation into the methods of protection of the side chain functional groups in question.

In order to improve the solubility of asparagine and glutamine-containing fragments and also to minimize side reactions, protected carboxamides are preferred, e. g. bis(2,4-dimethoxybenzyl) amides[7] or *p*-methoxybenzyl amides[8]. The α-*p*-nitrobenzyl esters of *N*-Boc-aspartic acid and *N*-

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Abbreviation: Boc = t-butyloxycarbonyl.

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Boc-glutamic acid described here are suitable intermediates for the preparation of protected Boc-asparagine and Boc-glutamine. For the protection of the side-chain carboxylic acid groups of aspartic acid and glutamic acid containing fragments the *p*-nitrobenzyl group seems favourable because of its stability under the conditions for the acidolytic removal of a peptide[9] from the resin after solid-phase synthesis, and also during the subsequent N^{α}-tritylation[9].

The routes of synthesis for the eight different benzyl and *p*-nitrobenzyl monoesters of *N*-Boc-L-glutamic acid and *N*-Boc-L-aspartic acid and the data for the individual compounds are given in the Figure and the Table. Modifications of, or additions to procedures already described are illustrated by the following examples.

Experimental

N-Boc-γ-p-nitrobenzyl L-*glutamate, DMSO*

γ-Benzyl, L-*glutamate*[10] (30.0 g, 127 mmol) was added gradually in small portions with stirring to fuming *nitric acid* (50 m*l*), precooled to −15°C by immersion in an ice-salt bath. After the addition, which took 15 min, the mixture was left to stand for 45 min at 0°C, and then poured on to ice (ca. 100 g), and the pH adjusted to 9—10 by addition of concentrated aqueous ammonia (85 m*l*). After acidification to pH 5—6 with glacial acetic acid (2—3 m*l*), the mixture was left to stand at +5°C for 1 h. Filtration and recrystallization from water (800 m*l*) produced faintly yellow crystals (20 g, 56%) of γ-*p*-nitrobenzyl L-glutamate m. p. 165—166°C (Literature[13] yield 20%, m. p. [corr.] 171°C).

In a 3-*l* round-bottomed flask γ-*p*-nitrobenzyl L-glutamate (85 g, 302 mmol) was mixed with dimethylsulfoxide (2 *l*), triethylamine (110 m*l*, 790 mmol) and Boc-azide (60 m*l*, 420 mmol). After being stirred for 16 h at room temperature the clear, orange-red liquid was diluted to 6 *l* with water, and excess Boc-azide removed by ether-extraction. After acidification to pH 3 with citric acid, extraction with ethyl acetate, back-extraction of the combined organic phases with water, drying over magnesium sulfate and concentration of the filtrate *in vacuo* produced a syrup (86 g (75%); literature[14] yield > 100%, uncharacterized), which crystallized on addition of dimethylsulfoxide (20 m*l*); m. p. 92—94°C. Recrystallization from ethyl acetate afforded a faintly yellow, somewhat hygroscopic crystalline product (78 g, 56%) of m. p. 104 to 105°C.

Neutralization equivalent: found 489; calc. 461. Sulfoxide titration equivalent[18]: found 478; calc. 461. Proof of *para* nitration: the yellow, crystalline residue isolated by ether extraction of an alkaline hydrolyzate had m. p. 89—91°C, and a mixed melting point with authentic *p*-nitrobenzyl alcohol showed no depression.

*N-Boc-α-p-nitrobenzyl-β-dicyclohexylammonium-*L-*aspartate*

N-Boc-L-aspartic anhydride[16] (24.7 g, 115 mmol) was dissolved in dimethylformamide (90 m*l*) and newly recrystallized *p*-nitrobenzyl alcohol (19.8 g, 129 mmol) was added to the clear solution. After 30 min of stirring a solution of dicyclohexylamine (24 m*l*, 122 mmol) in dry ether (50 m*l*) was added dropwise to the reaction mixture. In a short time a solid had started to form and after 40 min the mixture had solidified. It was then diluted with dry ether (1.5 *l*), stirred for 2 h and stored overnight at 4°C. The solid was filtered, washed thoroughly with dry ether and dried *in vacuo*. Yield 41.5 g (66%) of m. p. 165—167°C. (Literature[16] yield 80% of m. p. 160—162°C).

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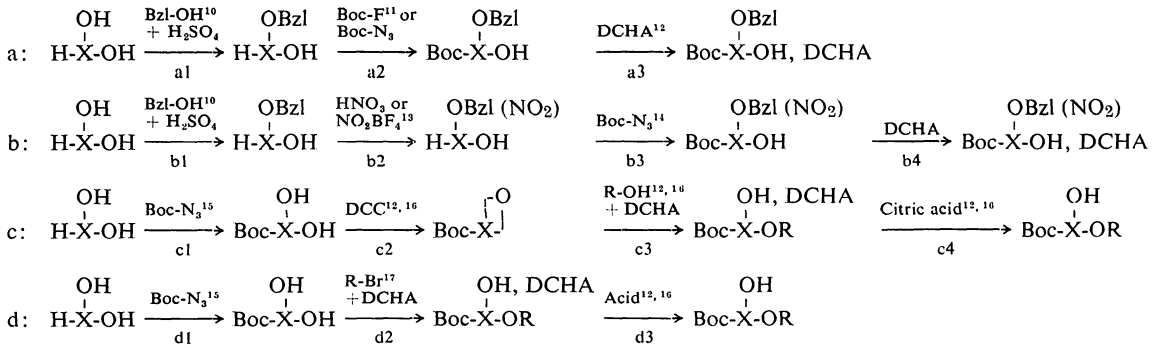

Figure. Routes of synthesis of benzyl and *p*-nitrobenzyl monoesters of *N*-Boc-L-glutamic acid and *N*-Boc-L-aspartic acid. Boc = t-butyloxycarbonyl; Bzl = benzyl; DCHA = dicyclohexylamine; DCC = dicyclohexylcarbodiimide; H-X-OH = glutamic or aspartic acid.

Table. Synthesis, melting points, optical rotation and microanalysis of benzyl and *p*-nitrobenzyl monester of *N*-Boc-L-glutamic and *N*-Boc-L-aspartic acid. All melting points are uncorrected. DMF = dimethylformamide, THF = tetrahydrofuran, MeOH = methanol, AcOH = acetic acid, DMSO = dimethyl sulfoxide. For other abbreviations see legend to the Figure.

N-Boc-amino acid	Synthesis				Optical rotation					Microanalysis (upper values calc. lower values found)				Literature
	Method	Yield [%]	Solvent	M. p. [°C]	$[\alpha]^t_{578}$	$[\alpha]^t_{589}$	*t* [°C]	*c*	Solvent	C	H	N	O	
OH, DCHA -Asp-OBzl	c3	44	DMF	127–128	– 7.2°	– 6.6°	22	1	DMF	66.6	8.8	5.6	19.0	
										66.6	8.9	5.5	19.0	
OH, DCHA -Asp-OBzl (NO_2)	c3	66	DMF	165–167	– 11.4°	– 11.4°	22	1	DMF					
	c3	80[a]	THF	166–167	—	– 11.7°	25	1	DMF					SCHRÖDER and KLIEGER[16]
OH -Asp-OBzl	c4	80		96–97	– 24.2°	– 22.6°	22	1	MeOH	59.4	6.6	4.3	29.7	
										59.1	6.6	4.3	29.9	
OH -Asp-OBzl (NO_2)	c4	72		128–129	– 24.0°	– 22.5°	22	1	MeOH	52.2	5.5	7.6		
	c4			135–136		– 8.5°	25	1	MeOH	52.4	5.6	7.8		SCHRÖDER and KLIEGER[16]

	a2	75	DMSO	101	+ 8.7°	+ 8.5°	22	1	AcOH					
OBzl						+ 28.4°	26	2	MeOH					
-Asp-OH	—	—	—	99		+ 36.5°	26	2	MeOH					BAYER *et al.*[19]
	a2	86[b]	Dioxan/H_2O pH 8.8	95—97	+ 7.1°		—	1	AcOH					SCHNABEL *et al.*[11]
	a2	75	DMF	103		+ 10.2°	22	2	AcOH					POLZHOFER[17]
OBzl (NO_2)														
-Asp-OH	b3	58	DMSO	130—132	+ 1.7°	+ 2.4°	25	1	MeOH					BRUNFELDT and HALSTRØM[9]
OBzl	a2	69	DMSO	55—57[c]	— 5.9°	— 5.2°	22	1	AcOH					
-Glu-OH	a2	50	Dioxan/H_2O pH 8.6	58—59	— 5.8°	— 5.6°	25	1	AcOH					HANSEN[20]
OH, DCHA														
-Glu-OBzl	d2	42	DMF	170—172		— 18.9°	22	1	MeOH					
	c2, 3	30	THF	172		— 19.2°	25	1	MeOH					SCHRÖDER and KLIEGER[12]
OH, DCHA														
-Glu-OBzl (NO_2)	c3	43	DMF	175—176	— 11.0°	— 10.2°	22	1	MeOH					
	c3	50[a]	THF	171—172		— 10.6°	25	1	MeOH					SCHRÖDER and KLIEGER[12]
OH														
-Glu-OBzl	c4	66		94—95	— 32.3°	— 30.5°	22	1	MeOH					
	c4	81		93—94		— 30.2°	25	1	MeOH					SCHRÖDER and KLIEGER[12]
OH										53.4	5.8	7.3		
-Glu-OBzl (NO_2)	c4	70		99—100	— 21.3°	— 20.1°	22	1	MeOH	53.6	5.9	7.2		
	a2, 3	35	Dioxan/H_2O pH 8.8	138—139	+ 12.3°	+ 11.8°	22	1	MeOH					
OBzl														
-Glu-OH, DCHA	a2, 3	30[a]	H_2O, MgO	138—139		+ 11.9°	25	1	MeOH					SCHRÖDER and KLIEGER[12]
	a2, 3	60[b]	Dioxan/H_2O pH 8.8	140—142	— 3.9°		22	1	AcOH					SCHNABEL *et al.*[11]
	a2, 3	71	DMF	138—139		+ 11.6°	13	1	MeOH					POLZHOFER[21]
	b3, 4	15	Dioxan/H_2O pH 8.7	144—146	+ 11.1°	+ 10.7°	25	2	DMF					BRUNFELDT and HALSTRØM[9]
OBzl (NO_2)														
-Glu-OH, DCHA	b3, 4	30	DMSO	146—148	+ 11.2°	+ 10.7°	25	2	DMF					BRUNFELDT and HALSTRØM[9]
OBzl (NO_2)														
-Glu-OH, DMSO	b3	57	DMSO	104—105	— 8.0°	— 7.2°	22	1	MeOH	49.6	6.1	6.1	31.3[d]	
										49.5	6.1	6.2	31.1	

[a]) Yield is given for crude product only. [b]) Boc fluoride was used instead of Boc-azide. [c]) Crystallization by trituration with petroleum ether was possible only after extraction into sodium hydrogencarbonate solution, acidification and back-extraction into ethyl acetate. [d]) Microanlysis: $S_{found} = 6.8\,\%$. $S_{calc.} = 7.0\,\%$.