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Reactions with N-(1-benzotriazolylcarbonyl)-amino acids. IV. The use of N-(1-benzotriazolylcarbonyl)-amino acid derivates in peptide synthesis

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The use of the 1-benzotriazolylcarbonyl-(Btc)-group as an N-protecting and N-activating group in the synthesis of peptides was investigated. Removal of the Btc group from N-Btc-amino acids, their esters and amides under acidic conditions is possible, but has no advantages over removal of benzyloxycarbonyl-(Z)-group. N-Btc-amino acid esters react with Z-amino acids or Z-dipeptides yielding Z-dipeptide and Z-tripeptide esters, respectively. This process is accompanied with separation of benzotriazole and CO_2 . Advantages and disadvantages of this method of peptide bond formation are discussed.

N-(1-Benzotriazolylcarbonyl)-(Btc)-amino acids¹ (1) have been previously used in peptide synthesis². The peptide bond formation was achieved by means of 1-benzotriazolylcarbonyl group as both by a N-protecting and C-activating group. In this paper we report new methods of peptide bond formation using the Btc group, either as a N-protecting or N-activating group.

The Btc Group as an N-Protecting Group in the Peptide Bond Formation

The synthesis of N-(Btc)-amino acid amides (3) from 1 and their alkaline hydrolysis to hydantoins as the only products has already been reported³. N-Btc-amino acids (1), amides 3 and the here described N-Btc-amino acid esters 4 are quite stable in diluted hydrochloric acid. Acidic hydrolysis of the Btc group is observed after a longer treatment (several hours at room temperature). The degree of hydrolysis is higher at increased temperatures, but heating also enhances formation of hydantoins, e.g. Btc-D,L-phenylglycine benzyl amide (3d) in acetone/5% HCl (5 hrs, 60°C) gives D,L-phenylglycine benzyl amide (6d) and 3-benzyl-5-phenyl-hydantoin (5a) in 1:1 ratio. When the N-Btc-D,L-phenylglycine butyl amide (3b) is refluxed in xylene for 8 hrs, it cycles to 3-butyl-5-phenylhydantoin (5b) (60% yield). This reaction is in agreement with the known dissociation of carbamoyl benzotriazole to benzotriazole and isocyanates⁴ and their cyclization to hydantoins⁵:

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R-CH-COOH
$$\frac{SOC1}{2}$$
, R-CH-COC1 $\frac{R'NH}{2}$ $\frac{R-CH-CONHR'}{NHBte}$ $\frac{R'NH}{3}$ $\frac{R-CH-CONHR'}{NHBte}$ $\frac{R'OH}{3}$ $\frac{R-CH-CONR'}{NHBte}$ $\frac{A}{3}$ $\frac{A}{3}$

D	D.		compo	und	
R	R ′	1	3 and 6	4	5
p-OH-C ₆ H ₅ CH ₂		a			
CH ₃	c-C ₆ H ₁₁	-	a		
C ₆ H ₅	C ₄ H ₉		b		b
C ₆ H ₅	c-C ₆ H ₁₁		С		
C ₆ H ₅	$C_6H_5CH_2$		d		a
C ₆ H ₅ CH ₂	$C_6H_5CH_2$		е		
C ₆ H ₅ CH ₂	CH ₃ SCH ₂ CH ₂ CH-		f		
	ĊH ₂ OI	-[I		
Н	CH ₃	b)		a	
H	$C_6H_5CH_2$	H off		b	
CH ₃ SCH ₂ CH ₂	C ₂ H ₅			С	
C ₆ H ₅ CH ₂	CH ₃			d	
C ₆ H ₅ CH ₂	$C_6H_5CH_2$			е	С
C_6H_5	CH ₃			f	
CH ₃	$C_6H_5CH_2$			g	
(CH ₃) ₂ CHCH ₂	$C_6H_5CH_2$			h	

Acidolysis of Btc group is easier in trifluoroacetic acid (TFA): N-Btc-amino acid amides 3e, 3f and N-Btc-L-tyrosine (1a), after standing in TFA at room temperature for 24 hrs, produce amino acid amides 6e and 6f, and tyrosine, respectively. Table I shows the results of acidolysis of some N-Btc-amino acid derivatives.

Catalytic hydrogenation, which is a well known deblocking metod for Z- protective group, proved to be unsuccessful in the case of Btc group: no hydrogen uptake occurred when N-Btc-amino acid amides where hydrogenated on Pd(5%)/C, $Pd(5\%)/BaSO_4$ in ethanol or ethyl acetate.

The general conclusion is that the *N*-Btc group, as an N-protecting group, has no advantages over benzyloxycarbonyl-(Z)- and other alkyloxycarbonyl groups.

The Btc Group as an N-Activating Group in the Peptide Bond Formation

It was previously confirmed that 1-benzotriazole carboxylic acid amides (BtcNHR, "active ureas"), like some other, by Staab⁶ investigated carbamoylazoles, dissociate into benzotriazole and isocyanates⁴. It is also known that N-1- imidazolylcarbonyl) and N-[1-(1,2,4-triazolyl)-carbonyl]-amino acid esters $\bf a$ at higher temperature dissociate into α -isocyanate esters $\bf b$ and the corresponding azole. Thus formed α -isocyanate esters react with N-protected amino acids (e.g. Z-amino acids) forming N-protected dipeptide esters $\bf c$.

TABLE I

Acidolysis of some N-Btc-amino acid derivates

		Reaction	conditio	ns	
	Compound	Acid	Temp.	React. time (h)	Product*
1a	Btc-L-Tyr-OH	TFA	20	24.0	H-L-Tyr-OH
3a	Btc-D,L-Ala-NHc-C ₆ H ₁₁	acetone/HCl	60	10.5	H-D,L-Ala-NHc-C ₆ H ₁₁ (6a)
3b	Btc-D,L-Pgly-NHC4H9	п	11	3.5	H-D,L-Pgly-NHC4H9 (6b)
3c	Btc-D,L-Pgly-NHc-C ₆ H ₁₁	n	"	21.0	H-D,L-Pgly-NHc-C ₆ H ₁₁ (6c)
3d	Btc-D,L-Pgly-NHCH ₂ -C ₆ H ₅	***		5.0	H-D,L-Pgly-NHCH ₂ C ₆ H ₅ (6d) + hydantoin (5a)
3e	Btc-L-Phe-NHCH ₂ -C ₆ H ₅	a queen sel	"	3.2	H-L-Phe-NHCH ₂ -C ₆ H ₅ (6e) + hydantoin (5c)
3e	alsona paragraphic	TFA	20	24.0	H-L-Phe-NHCH ₂ C ₆ H ₅ (6e)
3f	Btc-L-Phe-L-methioninol	"	11	"	H-L-Phe-L-methioninol (6f)
4a	Btc-Gly-OCH ₃	dioxane/HCl	100	2.0	H-Gly-OCH ₃ + H-Gly-OH
"	interest head days to	TFA	72	0.66	an aponto and said m
4b	Btc-Gly-OCH ₂ C ₆ H ₅	acetone/HCl	60	16.0	H-Gly-OCH ₂ C ₆ H ₅ +H-Gly-OH
11	imp boomally odda slo	dioxane/HCl	100	8.0	ou gambraig auw w)
11	CENTRALED LOURNIES	TFA	20	120.0	Brown order All Core
"	comballing half resemble	"	72	0.5	evintes and letter some observer
4d	Btc-L-Phe-OCH ₃	dioxane/HCl	100	1.25	H-L-Phe-OCH3+H-L-Phe-OH
4e	Btc-L-Phe-OCH ₂ -C ₆ H ₅	"	11	2.0	

^{*} Hydantoins were not always isolated. For spot detection on TLC, a mixture of H₂SO₄ and MeOH (1:9)/120°C was used.

The main disadvantage of this method (Goldschmidt⁷ and Gante^{8,9}) is the requirement for previous preparation of unstable and toxic α -isocyanate esters.

We synthesized N-Btc-amino acid esters 4 and used them for the preparation of low peptides. Btc esters 4, unlike esters a, can be prepared directly, since benzotriazole is an azole able to form azolylcarbonyl chloride⁴. N-Btc-amino acid esters 4 were synthesized in two ways:

a) Btc-NH-CH(R)-COOH
$$\begin{array}{c} \text{COCl}_2 \\ \text{1} \\ \text{R'OH} \\ \text{Btc-NH-CH(R)-COOR'} \\ \textbf{4a-h} \\ \text{b) Btc-Cl} + \text{HCl} \cdot \text{H}_2\text{N-CH(R)-COOR'} \\ \end{array}$$

Both methods gave esters 4 of similar purity and in comparable yields. In most cases, besides esters 4, a small amount (6-14%) of ureido esters 8 was formed. The formation of ureido compounds 8 as by-products in the synthesis of t-amyloxycarbonyl-(AOC)-amino acid esters from amino acid esters and AOC-Cl in the presence of one equivalent of base [triethylamine, pyridine, N-ethylmorpholine or N,N-diethylglycine ethyl ester (DEG)] has been described in literature. The lowest percentage of ureido esters was obtained in reactions with DEG.

Following these literature data two experiments were performed: the synthesis of N-Btc-D-phenylglycine methyl ester (4f) by method b with NMM as hydrogen chloride acceptor and with DEG, respectively. In both experiments the same amount (6%) of N,N'- carbonyl-bis(D-phenylglycine methyl ester) was formed. No further attempts to prevent formation of ureido esters 8 were made.

The reaction conditions, yields and properties of 4 are summarized in Table II. Compounds 4 are stable solids or oils. Their structure has been confirmed by CHN analysis and IR spectroscopy. Characteristic absorptions in IR spectra are: 3410-3340 (NH), 1755-1725 (CO) and 1530-1500 cm⁻¹ (amide II). In accordance with previous results ^{1,4,6}, the carbonyl group bonded with benzotriazole absorbs at 1750 and ester carbonyl at 1725 cm⁻¹. In most cases, these two absorptions are not separated, but they appear as one broad absorption band.

N-Btc-amino acid esters reacted in an equimolar ratio with the carboxylic group of N-Z-protected amino acids or dipeptides, yielding N-Z-di- 9 and tripeptide esters 10, respectively:

Z-NH-CH(R)-COOH + Btc-NH-CH(R)-COOR'
$$\frac{-BtH,-CO_2}{4}$$

Z-NH-CH(R)-CONH-CH(R)-COOR' $9a-g$

The best results were obtained by heating the reactants for several hours in waterless xylene at 140°C. For isolation and purification of products 9 and 10, recrystallization and column chromatography were used.

Reaction times, yields and the properties of the N-Z-di- and tripeptide esters are summarized in Table III.

The described method of peptide bond formation is a modification of the isocyanate method. This method offers some advantages: the N-Btc- amino acid esters may be considered as stable and non-volatile isocyanates and are, therefore, easier and less dangerous to handle than the α -isocyanate esters. This approach is particularly convenient in the synthesis of homologous compounds. Our method has no advantages over the other known methods of peptide bond formation. 11,12

EXPERIMENTAL

All melting points are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Specific rotation data were taken on the "Opton" polarimeter. For thin-layer chromatography, silica gel sheets Kieselgel 60 F₂₅₄ "Merck" were used. Solvent systems were benzene/ethylacetate 9:1, 7:3 or 1:1 and chloroform/methanol 9:1. For spot detection ninhydrin, iodine or a mixture of methanol and conc. sulfuric acid 9:1 were used. Column chromatography was performed on silica gel 0.063-0.200 mm. The N-Btc-amino acids, their chlorides, ^{1,3} H-Gly-OBzl, ²³ H-L-Met- OEt, ²⁴ H-D-Pgly-OMe²⁵ and H-L-Phe-OBzl²⁶ were synthesized according to the literature. Z-L-Phe-OH, Z-Gly-L-Phe-OH and Z-L-Leu-L-Ala-OH were purchased from "Fluka". Z-L-Tyr(Bzl)-Gly-OH was prepared by saponification of the methyl ester following the method of Wünsch. ²⁷

D,L-Alanine Cyclohexylamide (H-D,L-Ala-NH-c-C₆H₁₁) (6a)

A suspension of 2.21 g (7 mmol) of Btc- $D_{s}L$ -Ala-NH-c- $C_{6}H_{11}$ (3a) in 30 ml acetone and 30 ml 5% HCl was refluxed for 10.5 hrs. Acetone was removed in vacuo and the water solution was extracted several times with chloroform (until all of benzotriazole was removed). The aqueous layer was evaporated in vacuo. The crude product 6a hydrochloride (1.45 g, 100%) was recrystallized from methanol/ether. m.p. 239-241°C (Lit. 28 238-240°C).

IR(KBr): vmax 3500-2500, 3280, 1675, 1545 cm⁻¹.

D,L-Phenylglycine Butylamide (H-D,L-Pgly-NHC4H9) (6b)

From 2.11 g (6 mmol) Btc- D_1L -Pgly-NHC₄H₉ (3b) 0.92 g (63%) 6b HCl was prepared (an analogous procedure to that for 6a). m.p. of hydrochloride 64-65°C.

IR(KBr): vmax 3660-2400, 1665, 1555, 1475 cm⁻¹.

TABLE II

N-(1-benzotriazolylcarbonyl)-amino acid esters (4a-h)

REACTANTS	ANTS	METHOD	SOLVENT	LIME / P	PRODUCT	KIECD / %	m. p. (°C)	SOLVENT FOR RECRYST.	IR (KBr or film) (cm ⁻¹)
Gly-OMe·HCl	BtcCl+NMM	В	benzene	72	Btc-Gly-OMe (4a)	85	133-134	acetone/ water	3370, 1745, 1530
Btc-Gly-Cl	BzIOH+TEA	A	benzene	1	Die Gle Obel (4b)	52	150 151	acetone,	3360 1755 1730 1530
Gly-OBzl-HCl	BtcCl+NMM	В	dioxane	1	DIC-OIY-ODZI (40)	46	101-001	methanol	3300, 1733, 1730, 1330
L-Met-OEt·HCI	BtcCl+NMM	В	dioxane	1.5	Btc-L-Met-OEt (4c)	81	lio	1	3350, 1740, 1520
Btc-L-Phe-Cl	МеОН	A	methanol	0.75	Dto I Dho OMo (44)	66			3370 1735 1500
$L ext{-Phe-OMe-HCl}$	BtcCl+NMM	В	dioxane	2	DIC-T-1 IIC-OME (4a)	77	100		3370, 1723, 1300
Btc- L -Phe- Cl	BzlOH+TEA	A	penzene	0.75	Dto I Dho Obel (40)	64			3400 1740 1515
L-Phe-OBzl·HCl	BtcCl+NMM	В	dioxane	1.5	DIC-E-1 IIC-OB4 (4e)	82	TO .		2400, 1/40, 1212
Btc-D-Pgly-Cl	МеОН	A	methanol	0.5		94		benzene/	
$D ext{-Pgly-OMe-HCl}$	BtcCl+NMM	В	dioxane	2	Btc-L-Pgly-OMe (4f)	87	72-75	petrol-	3410, 1740, 1500
$D ext{-Pgly-OMe-HCI}$	BtcCl+DEG	В	dioxane	2		80		ether	
Btc- D,L -Ala-Cl	BzIOH+TEA	A	penzene	0.25	Btc-D,L-Ala-OBzl (4g)	72	70-71	ether/ petrol- ether	3340, 1755, 1730, 1525
Btc-L-Leu-Cl	BzIOH+TEA	A	benzene	0.75	Btc-L-Leu-OBzl (4h)	75	62-64	methanol	3320, 1725, 1505

TABLE III

Z-dipeptide- (9a-g) and Z-tripeptide esters (10a-c)

					SOI VENT	7		
COMPOUND	LIWE	YIELD (%)	ш. р. (°С)	Lit. m. p. (°C)	FOR RECRYST.	$[lpha]_{ m D}^{20}$	Lit. [a] ²⁴	IR (KBr) (cm ⁻¹)
Z-Gly-Gly-OMe (9a)	6.5	09	63-65	63-65 ¹³ 66.5-67.5 ¹⁴	ethyl acetate/ petrolether	1	2	3320, 1740, 1690, 1660, 1530
Z-Gly-Gly-OBzl (9b)	7.0	55	110-112	$\frac{110^{15}}{111-112^{16,17}}$	methanol/ water			3380, 1740, 1710, 1655, 1530
Z- L -Phe-Gly-OBzl (9c)	4.5	75	133-134	$130-131^{17} \\ 135.5-137.5^{18} \\ 138^{15}$	ethyl acetate/ petrolether	* -10.7° (c 0.1,AcOH)	* -9.2° (c 2, AcOH) ¹⁸	3300, 1740, 1695, 1660, 1540
Z-L-Tyr(Bzl)-Gly-OMe (9d)	10.0	99	127-128	126-127 ¹⁹	ethyl acetate	* -24.6° (c 0.76,DMF)	-23.1° (c 0.96,DMF) ¹⁹	3300, 1750, 1695, 1655, 1540
Z-L-Phe-L-Met-OEt (9e)	4.5	51	121-124		ethyl acetate/ petrolether	+11.7° (c 0.5,CHCl3)		3300, 1725, 1690, 1660, 1530
Z-Gly-L-Phe-OBzl (9f)	13.0	89	69-89	74 ²⁰	,	-4.8° (c 1, EtOH)	** -4.5° (c 1, EtOH) ²⁰	3390, 3320, 1730, 1705, 1655, 1530
Z - L -Phe- L -Phe-OBzl $(9\mathbf{g})$	8.5	75	152-153	$149-150^{21} \\ 156-157^{20}$	methanol	(c 2, CHCl ₃)	$+9.1^{\circ}$ (c 2, CHCl ₃) ²⁰	3300, 1735, 1700, 1655, 1550
Z- L -Tyr(Bzl)-Gly-Gly-OMe (10a)	12.0	59	152-155	161-163 ²²	ethyl acetate	** -15° (c 0.9,50% THF)	** -18.8° (c 0.9,50% THF) ²²	3400, 1740, 1715, 1675, 1500
Z-Gly-L-Phe-L-Met-OEt (10b)	12.0	53	98-100	1	ethyl acetate/ petrolether	-9.7° (c 0.89, EtOH)		3250, 1720, 1685, 1650, 1515
Z-L-Leu-L-Ala-D-Pgly-OMe (10c)	11.0	61	176-178		methanol/ water	** -82° (c 1, CHCl3)	•	3270, 1735, 1685, 1640, 1520

* $t = 23^{\circ}$ C ** $t = 25^{\circ}$ C

N-Acetylderivate: A water solution of **6b** hydrochloride was made alkaline with dil. NaOH (pH 9), the free base **6b** was extracted with chloroform and acetylired with acetanhydride. The crude product was chromatographed (silica gel/chloroform + 5% methanol) and recrystallized from ethanol. m.p. 171-173°C.

IR(KBr): νmax 3290, 1635, 1535 cm⁻¹.

C₁₄H₂₀N₂O₂ (248.33) calc. N 11.28 found 11.03

D_{L} -Phenylglycine Cyclohexylamide (H- D_{L} -Pgly-NH-c- C_{6} H₁₁) (6c)

0.16 g (84%) 6c was prepared from 0.26 g (0.7 mmol) Btc-D,L- Pgly-NH-c-C₆H₁₁ (3e) in 3 ml acetone and 3 ml 5% HCl. m.p. 121-124°C.

IR(KBr): vmax 3500-2500, 1655, 1570, 1510 cm⁻¹.

C₁₄H₂₁ClN₂O (268.78) calc. C 62.56 H 7.88 N 10.42 found 62.10 7.71 10.01

D,L-Phenylglycine Benzylamide (H-D,L-Pgly-NHCH₂C₆H₅) (6d) and 3-Benzyl-5-phenylhydantoin (5a)

2.69 g (7 mmol) Btc-D,L-Pgly-NHCH₂C₆H₅) (3d) was refluxed for 5 hrs in 30 ml acetone and 30 ml 5% HCl. After removal of acetone, 3-benzyl-5-phenylhydantoin (5a) crystallized (0.70 g, 38%). m.p. 171- 174°C.

IR(KBr): vmax 3500, 1770, 1715 cm⁻¹.

C₁₆H₁₃N₂O₂ (265.29) calc. C 72.44 H 4.94 N 10.56 found 72.37 5.21 10.43

 1 H NMR (CDCl₃) **d**(ppm): 4.67(s,2H,CH₂); 5.03(d,1H,CH); 6.14(s,1H,NH); 7.30(m,10 H_{arom}.).

The filtrate was made alkaline with dil.NaOH (pH 9) and amide 6d extracted with benzene. Yield: 0.96 g (57%). m.p. 54-56°C.

IR(KBr): vmax 3330, 3290, 1645, 1515 cm⁻¹.

C₁₅H₁₆N₂O (240.31) calc. C 74.97 H 6.71 N 11.66 found 74.84 6.98 11.75

6d was transformed into hydrochloride by means of HCl/MeOH. m.p. 148-151°C. IR(KBr): ν max 3400-2500, 1665, 1560, 1485 cm⁻¹.

L-Phenylalanine Benzylamide (H-L-Phe-NHCH₂ C_6H_5) (**6e**) and 3,5-dibenzylhydantoin (**5c**)

a) 3.57 g (9 mmol) Btc-*L*-Phe-NHCH₂C₆H₅) (3e) was refluxed in 100 ml acetone and 45 ml 5% HCl for 4 hrs. After removing acetone in vacuo, 3,5-dibenzyl-hydantoin (5c) crystallized (1.02 g, 40%; IR, $[\alpha]_D^{20}$ and m.p. was identical to test substance³). The filtrate was extracted several times with ethyl acetate in order to remove benzotriazole. The aqueous layer was evaporated under reduced pressure to give 0.86 g (33%) 6e HCl. m.p. 161-163°C. $[\alpha]_D^{20}$ +55.7° (c 0.78, methanol).

IR(KBr): vmax 3500-2500, 3410, 1665, 1535 cm⁻¹.

C₁₆H₁₉ClN₂O (290.79) calc. C 66.09 H 6.59 N 9.63 found 65.80 6.80 9.25 b) A solution of 0.50 g 3e in 20 ml trifluoroacetic acid (TFA) was left at room temperature for 24 hrs. After removing TFA in vacuo, the residue was dissolved in ethyl acetate and extracted several times with 2% NaOH (in order to remove BtH). The organic layer was washed with water, dried over Na₂SO₄ and evaporated to give 6e which was transformed into hydrochloride by means of HCl/2-propanol. Yield: 0.24 g (75%). IR spectrum, $[\alpha]_D^{20}$ and m.p. were identical to the product prepared following procedure a).

L-Phenylalanine-L-(1-hydroxymethyl-3-methylthiopropyl)amide (H-L-Phe-L-Methioninol) (6f)

A solution of 3.00 g (7 mmol) Btc-L-Phe-L methioninol (3f) in 15 ml TFA was left at room temperature for 24 hrs. After removing TFA in vacuo, the residue was dissolved in water, acidulated with HCl (pH 2) and extracted 18 times with benzene. The aqueous layer was evaporated and the crude 6f HCl was recrystallized from 2-propanol. Yield: 0.66 g (30%). IR spectrum, $[\alpha]_D^{20}$ and m.p. were identical to the test substance.²⁹ The mother liquor contained an additional amount of 6f inpured with benzotriazole.

3-Butyl-5-phenylhydantoin (5b)

0.30 g (0.85 mmol) Btc-p,L-Pgly-NHC₄H₉ (3b) was refluxed in 30 ml xylene for 8 hrs. After evaporating xylene under reduced pressure, the crude product 5b was chromatographed (silica gel, chloroform/ethyl acetate 8:2). Yield: 0.12 g (60.5%) 5b. m.p. 74-77°C (Lit.³ 72-76°C).

Acidolysis of Btc-amino Acid Esters

0.5 mmol of Btc-amino acid esters 4a-d in 10 ml trifluoro acetic acid was reacted (for the reaction conditions see Table I) until no starting ester could be detected on TLC (silica gel sheets, benzene/ethyl acetate 1:1, chloroform/methanol 9:1, butanol/acetic acid/water 4:1:1). In most cases, during this period, acidolysis of ester group occurred parallel to acidolysis of Btc, so the products were both amino acid esters and free amino acids, respectively. The reaction products were not isolated, but were identified on TLC (silice gel sheets in benzene/ethyl acetate 1:1) (Figure 1.)

- 1. Btc-Gly-OMe
- 2. Btc-Gly-OBzl
- 3. Btc-L-Phe-OMe
- 4. Btc-L-Phe-OBzl
- 5. benzotriazole
- 6. H-Gly-OMe
- 7. H-Gly-OBzl
- 8. H-L-Phe-OMe
- 9. H-L-Phe-OBzl
- 10. H-Gly-OH
- 11. H-L-Phe-OH

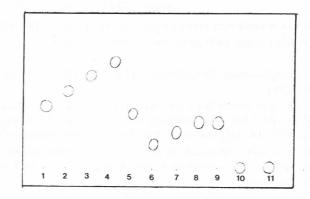


Figure 1. Acidolysis of Btc-amino acid esters: TLC of starting and final compounds

N-Btc-amino Acid Esters 4a-g: General Procedure:

Method A: to a solution of N-Btc-amino acid chloride (8 mmol) in 75 ml benzene, benzyl alcohol (0.86 g, 8 mmol) and triethylamine (0.81 g, 8 mmol) in 25 ml benzene were added dropwise. The reaction mixture was stirred at room temperature (see Table II) and extracted 3 times with a small amount of water. The organic layer was dried over sodium sulfate and evaporated. The methyl esters 4d i 4f were prepared by dissolving N-Btc-amino acid chlorides (6 mmol) in 20 ml methanol. After stirring at room temperature, methanol was evaporated in vacuo without heating.

N-Btc-Glycine benzyl ester (Btc-Gly-OBzl) (4b): The crude product was purified on a silica gel column (benzene/ethyl acetate 1:1).

N-Btc-L-Phenylalanine methyl ester (Btc-L-Phe-OMe) (4d): The crude product was purified on a silica gel column (benzene/ethyl acetate 8:2). $[\alpha]_D^{20} + 30.7^{\circ}$ (c 0.5, CHCl₃).

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C<sub>17</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub> (324.35) calc. C 62.96 H 4.97 N 17.27 found 62.72 5.18 17.53
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N-Btc-L-Phenylalanine benzyl ester (Btc-L-Phe-OBzl) (4e): The crude product was purified on a silica gel column (benzene/ethyl acetate 8:2). $[\alpha]_D^{20} + 9.5^{\circ}$ (c 2.6, CHCl₃).

```
C<sub>23</sub>H<sub>20</sub>N<sub>4</sub>O<sub>3</sub> (400.44) calc. C 68.99 H 5.03 N 13.99 found 69.23 5.23 14.11
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N-Btc-L-Phenylalanine methhyl ester (Btc-L-Pgly-OMe) (4f): The crude product was recrystallized from benzene/petroleum ether. $[\alpha]_D^{20}$ -127.2° (c 0.8, CHCl₃).

```
C<sub>16</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub> (310.31) calc. C 61.93 H 4.55 N 18.06 found 61.74 5.08 18.06
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N-Btc-D,L-Alanine benzyl ester (Btc-D,L-Ala-OBzl) (4g): The crude product was recrystal-lized from ether/petroleum ether.

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C<sub>17</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub> (324.35) calc. C 62.96 H 4.97 N 17.27 found 62.73 5.26 17.29
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 1 H NMR (CDCl₃) d(ppm): 1.65(d,3H,CH₃); 4.86(m,1H,CH); 5.25(s,2H,CH₂); 7.84(m,9H_{a-rom.} and 1H,NH).

N-Btc-L-Leucine benzyl ester (Btc-L-Leu-OBzl) (4h): The crude product was purified on a silica gel column (benzene/ethyl acetate 8:2). 2.19 g (75%) of 4h and 0.13 g (7%) of N,N'-carbonyl-bys(L-leucine benzyl ester) (8d) were eluated.

4d:
$$[\alpha]_D^{20}$$
 +4.43° (c 1.02, CHCl₃).

 1 H NMR (CDCl₃) d(ppm): 0.99(d,6H,2CH₃); 1.83(m,3H,CHCH₂CHCO); 4.80(m,1H,CHN); 5.23(s,2H,CH₂O); 7.82(m,9H_{arom.} and 1H,NH).

8d: IR(KBr): νmax 3320, 1710, 1625, 1500 cm⁻¹.

Method B: To a solution of 1.81 g (10 mmol) 1-benzotriazole carboxylic acid chloride in 20 ml dioxane or benzene, amino acid ester hydrochloride (10 mmol) was added. To this su-

spension, a solution of N-methyl-morpholine (NMM) (25 mmol) in 10 ml dioxane or benzene was added dropwise. The reaction mixture was stirred at room temperature (see Table II). N-Btc-amino acid esters were isolated as follows:

N-Btc-Glycine methyl ester (Btc-Gly-OMe) (4a): The reaction mixture was filtred and evaporated to dryness. The crude 4a was recrystallized from acetone /water.

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C_{10}H_{10}N_4O_3 (234.22) calc. C 51.28 H 4.30 N 23.92 found 50.99 4.58 23.82
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N-Btc-Glycine benzyl ester (Btc-Gly-OBzl) (4b): The reaction mixture was filtred and evaporated to dryness. The residue was chromatographed on silica gel column (benzene/ethyl acetate 9:1).

N-Btc-L-Methionine ethyl ester (Btc-L-Met-OEt) (4c): The same isolation procedure as for 4b. After 4c, 7% of N, N-carbonyl-bis(L-methionine ethyl ester) (8a) was eluated.

4c: $[\alpha]_D^{20}$ +8.9° (c 12.3, CHCl₃).

C₁₄H₁₈N₄O₃S (322.38) calc. C 52.16 H 5.63 N 17.38 found 52.39 5.63 17.56

8a: m.p. 87-89°C (Lit. 30 91°C).

8d: IR(KBr): vmax 3350, 1740, 1635, 1575 cm⁻¹.

 $N\text{-}Btc\text{-}L\text{-}Phenylalanine}$ methyl ester (Btc-L-Phe-OMe) (4d): The same isolation procedure as for 4c. 1.65 g (77%) 4d and 0.18 g (14%) N,N'-carbonyl-bis(L-phenylalanine methyl ester) (8b) was eluated.

8b: m.p. 157-159°C. Lit.¹⁰ m.p. 159.5-160.5°C. IR(KBr): *ν*max 3300, 1735, 1650, 1500 cm⁻¹.

N-Btc-L-Phenylalanine benzyl ester (Btc-L-Phe-OBzl) (4e): The same isolation procedure as for 4b.

N-Btc-D-Phenylglycine methyl ester (Btc-D-Pgly-OMe) (4f): The reaction mixture was filtred and evaporated to dryness. The residue was chromatographed on a silica gel column (benzene/ethyl acetate 8:2). Yield: 87% 4f and 6% of N,N'-carbonyl-bis(D-phenylglycine methyl ester) (8c).

8c: m.p. 189-191°C. Lit.³⁰ m.p. 185-186°C.

IR(KBr): vmax 3360, 1740, 1645, 1565 cm⁻¹

When the NMM was replaced with N,N-diethylglycine ethyl ester (DEG) as HCl acceptor 80% 4f and 6% 8c were isolated.

N-Z-Dipeptide (9a-g) and *N-Z-tripeptide esters* (10a-c): *General procedure*: A solution of N-Btc-amino acid ester (4 mmol) and N-Z-amino acid or *N-Z*-dipeptide (4 mmol) in 40 ml of waterless xylene was refluxed for 3-13 hrs (see Table III). The solvent was removed in vacuo. N-Z-di- or tripeptiden esters were isolated as follows:

N-Z-Glycyl-glycine methyl ester (Z-Gly-OMe) (9a): Unreacted Btc-Gly-OMe and BtH were separated from the mixture on a silica gel column using benzene/ethyl acetate 1:1 as eluent. Compound 9a was eluated with methanol and recrystallized from ethyl acetate/petroleum ether.

N-Z-Glycyl-glycine benzyl ester (Z-Gly-Gly-OBzl) (9b): Unreacted Btc-Gly-OBzl was removed by recrystallization from methanol. The pure compound 9b (43%) was precipitated from mother liquor by adding water. From filtrate, additional 12% of the product was isolated on a silica gel column (benzene/ethyl acetate 1:1).

N-Z-L-Phenylalanyl-glycine benzyl ester (Z-L-Phe-Gly-OBzl) (9c): Recrystallization from ether gave 46% of product 9c. Mother liquor was evaporated, the residue was dissolved in benzene and extracted 5 times with 15 ml ice-cold 2M NaOH. The organic layer was washed 2 times with a small amount of ice-cold water, dried and evaporated. In this way, additional 29% of compound 9c was obtained.

N-Z-O-Benzyl-L-tyrosyl-glycine methyl ester (Z-L-Tyr(Bzl)-Gly-OMe) (9d): Using column chromatography (benzene/ethyl acetate 7:3), unreacted Btc-Gly-OMe and a mixture of BtH and 9d were eluated. From this mixture 9d was isolated (45%) by recrystallization from ethyl acetate. Mother liquor was evaporated and purified on a silica gel column (chloroform/methanol 9.5:0.5). Additional 21% of compound 9d was obtained.

N-Z-L-Phenylalanyl-L-methionine ethyl ester (Z-L-Phe-L-Met-OEt) (9e): Using column chromatography (benzene/ethyl) acetate 8:2), 51% of compound 9e was isolated.

¹H NMR (CDCl₃) *d*(ppm): 1.26(t,3H,CH₃CH₂); 1,78(m,2H,CH₂CH₂S); 2.04(s,3H,CH₃S); 2.29(m,2H,CH₂S); 3.08(d,2H,CH₂CH); 4.17(q,2H,CH₃CH₂O); 4.55(m,2H,2CHCO); 5.09 (s,2H,CH₂O); 5.27(d,1H,CONH); 6.46(d,1H,NHCOO); 7.28(m,10H_{arom.}).

N-Z-Glycyl-L-phenylalanine benzyl ester (Z-Gly-L-Phe-OBzl) (9f): The oily residue was dissolved in benzene and extracted 5 times with 15 ml ice-cold 2M NaOH. The organic layer was washed twice with a small amount of ice-cold water, dried and evaporated. The crude product 9f was purified on a silica gel column (benzene/ethyl acetate 9:1).

N-Z-L-Phenylalanyl-L-phenylalanin benzyl ester (Z-L-Phe-L-Phe-OBzl) (9g): From the reaction mixture, 9g was partly crystallized (37%). The mother liquor was concentrated and additional product crystallized (23%). Xylene was evaporated and the oily residue worked up with methanol. Additional 15% of 9g precipitated. Yield: 75%.

N-Z-O-Benzyl-L-tyrosyl-glycyl-glycine methyl ester (Z-L-Tyr(Bzl)- Gly-Gly-OME) (10a): Unreacted Btc-Gly-OMe and BtH were separated from the mixture on a silica gel column using benzene/ethyl acetate 7:3 as eluent. Compound 10a was eluated with methanol. The crude product was washed with hot ethyl acetate.

N-Z-Glycyl-L-Phenylalanyl-L-methionine ethyl ester (Z-Gly-L-Phe-L-Met-OEt) (10b): Using column chromatography (benzene/ethyl acetate 1:1), unreacted Btc-Gly-OBzl, pure compound 10b and a mixture of 10b with BtH were eluated. This mixture was dissolved in benzene and extracted 3 times with 20 ml of ice-cold 0.5 M NaOH. The organic layer was washed twice with a small amount of ice-cold water, dried and evaporated. For analysis, 10b was recrystallized from ethyl acetate/petroleum ether.

¹H NMR (CDCl₃) *d*(ppm): 1.54(t,3H,CH₃); 2,02(s,3H,CH₃S); 2.27(m,4H,CH₂CH₂S); 3.04(d,2H,CH₂CHCO); 3.83(d,2H,CH₂CO); 4.13(q,2H,OCH₂CH₃); 4.65(m,2H,CHCO); 5.09 (s,2H,CH₂O); 5.67(m,1H,NH); 6.93(m,2H,2NH); 7.20(m,10H_{arom.}).

N-Z-L-Leucyl-L-alanyl-D-phenylglycine methyl ester (Z-L-Leu-L-Ala-D-Pgly-OMe) (10c): The same isolation procedure as for 10b.

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SAŽETAK

Reakcije s N-(1-benzotriazolilkarbonil)-aminokiselinama. IV. Upotreba derivata N-(1-benzotriazolilkarbonil)-aminokiselina u sintezi peptida B. Zorc, G. Karlović i I. Butula

Ispitana je mogućnost primjene 1-benzotriazolilkarbonilne (Btc) skupine kao zaštitne, odnosno aktivirajuće skupine u sintezi peptida. Nekoliko primjera acidolize amida i estera N-Btc-aminokiselina pokazuje da je otcjepljenje N-zaštitne Btc-skupine u principu moguće. Sintetizirani su esteri N-Btc-aminokiselina, koji u reakciji s benziloksikarbonil-(Z)-aminokiselinama, odnosno Z-dipeptidima daju, uz otcjepljenje benzotriazola i CO₂, odgovarajuće terminalno zaštićene di- i tripeptide. Diskutira se o nedostacima i prednostima ovih reakcija.