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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 acids[1] (**1**) have been previously used in peptide synthesis[2]. 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 reported[3]. *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 isocyanates[4] and their cyclization to hydantoins[5]:

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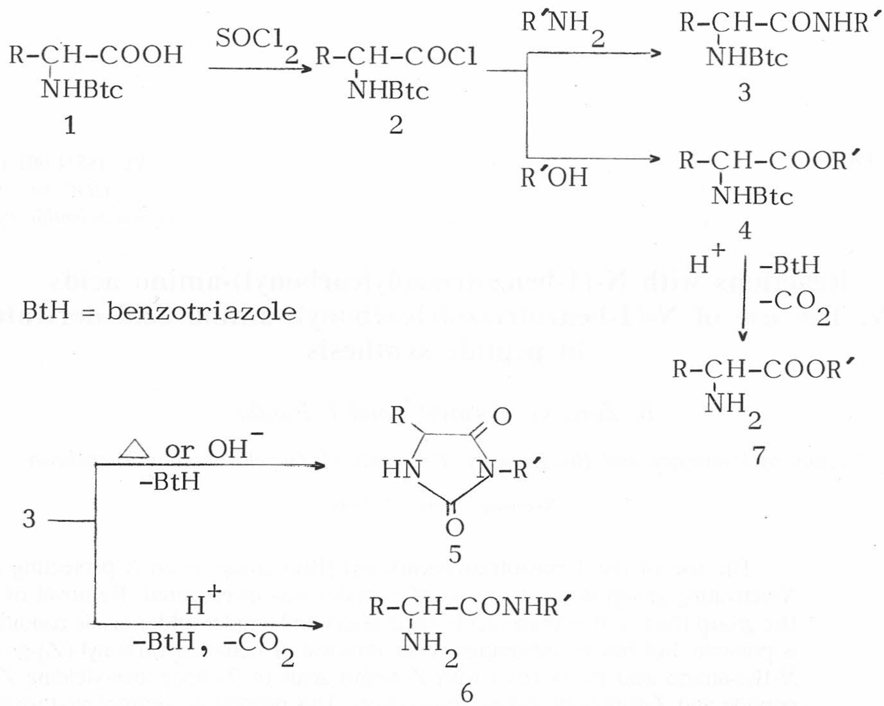
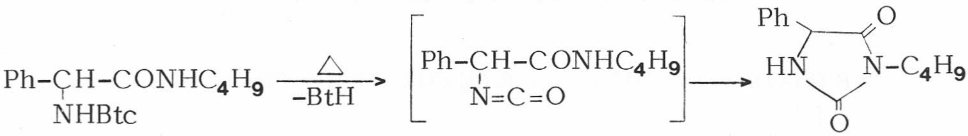


R	R′	compound 1	3 and 6	4	5
p-OH-$C_6H_5CH_2$	–	a			
CH_3	c-C_6H_{11}		a		
C_6H_5	C_4H_9		b		b
C_6H_5	c-C_6H_{11}		c		
C_6H_5	$C_6H_5CH_2$		d		a
$C_6H_5CH_2$	$C_6H_5CH_2$		e		
$C_6H_5CH_2$	$CH_3SCH_2CH_2CH(CH_2OH)-$		f		
H	CH_3			a	
H	$C_6H_5CH_2$			b	
$CH_3SCH_2CH_2$	C_2H_5			c	
$C_6H_5CH_2$	CH_3			d	
$C_6H_5CH_2$	$C_6H_5CH_2$			e	c
C_6H_5	CH_3			f	
CH_3	$C_6H_5CH_2$			g	
$(CH_3)_2CHCH_2$	$C_6H_5CH_2$			h	



3b 5b

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 Staab[6] investigated carbamoylazoles, dissociate into benzotriazole and isocyanates[4]. It is also known that *N*-1- imidazolylcarbonyl) and *N*-[1-(1,2,4-triazolyl)-carbonyl]-amino acid esters **a** at higher temperature dissociate into α-isocyanate esters **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 **c**.

OCN-CH(R)-COOR' $\xrightarrow{\text{Z-NHCH(R)COOH}}$ /Z-NH-CH(R)-COOCONH-CH(R)COOR'/
b
-azole ↑ ↓ +azole — -CO_2 ↓
azolyl-CONH-CH(R)-COOR' Z-NH-CH(R)CONH-CH(R)-COOR'
a **c**

TABLE I

Acidolysis of some N-Btc-amino acid derivates

$$\underset{\text{NHBtc}}{\underset{|}{\text{R-CH-COX}}} \xrightarrow{H^+} \underset{\text{NH}_2}{\underset{|}{\text{R-CH-COX}}} + \text{BtH} + \text{CO}_2$$

X=OH(**1a**), NHR'(**3a-f**), OR'(**4a-e**)

	Compound	Reaction conditions			Product*
		Acid	Temp. (°C)	React. time (h)	
1a	Btc-*L*-Tyr-OH	TFA	20	24.0	H-*L*-Tyr-OH
3a	Btc-*D,L*-Ala-NHc-C_6H_{11}	acetone/HCl	60	10.5	H-***D,L***-Ala-NHc-C_6H_{11} (**6a**)
3b	Btc-*D,L*-Pgly-NHC_4H_9	"	"	3.5	H-***D,L***-Pgly-NHC_4H_9 (**6b**)
3c	Btc-*D,L*-Pgly-NHc-C_6H_{11}	"	"	21.0	H-***D,L***-Pgly-NHc-C_6H_{11} (**6c**)
3d	Btc-*D,L*-Pgly-$NHCH_2$-C_6H_5	"	"	5.0	H-***D,L***-Pgly-$NHCH_2C_6H_5$ (**6d**) + hydantoin (**5a**)
3e	Btc-*L*-Phe-$NHCH_2$-C_6H_5	"	"	3.2	H-*L*-Phe-$NHCH_2$-C_6H_5 (**6e**) + hydantoin (**5c**)
3e	"	TFA	20	24.0	H-*L*-Phe-$NHCH_2C_6H_5$(**6e**)
3f	Btc-*L*-Phe-*L*-methioninol	"	"	"	H-*L*-Phe-*L*-methioninol (**6f**)
4a	Btc-Gly-OCH_3	dioxane/HCl	100	2.0	H-Gly-OCH_3 + H-Gly-OH
"	"	TFA	72	0.66	" "
4b	Btc-Gly-$OCH_2C_6H_5$	acetone/HCl	60	16.0	H-Gly-$OCH_2C_6H_5$+H-Gly-OH
"	"	dioxane/HCl	100	8.0	" "
"	"	TFA	20	120.0	" "
"	"	"	72	0.5	" "
4d	Btc-*L*-Phe-OCH_3	dioxane/HCl	100	1.25	H-*L*-Phe-OCH_3+H-*L*-Phe-OH
4e	Btc-*L*-Phe-OCH_2-C_6H_5	"	"	2.0	" "

* Hydantoins were not always isolated. For spot detection on TLC, a mixture of H_2SO_4 and MeOH (1:9)/120°C was used.

The main disadvantage of this method (Goldschmidt[7] 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 chloride[4]. N-Btc-amino acid esters **4** were synthesized in two ways:

a) Btc-NH-CH(R)-COOH $\xrightarrow{COCl_2}$ Btc-NH-CH(R)-COCl
1

$\downarrow$ R'OH

Btc-NH-CH(R)-COOR'
4a-h

$\uparrow$ NMM

b) Btc-Cl + $HCl \cdot H_2N$-CH(R)-COOR'

NH-CH(R)-COOR'
|
CO
|
NH-CH(R)-COOR'
8a-d

8	R	R'
a	$CH_3SCH_2CH_2$	C_2H_5
b	$C_6H_5CH_2$	CH_3
c	C_6H_5	CH_3
d	$(CH_3)_2CHCH_2$	$C_6H_5CH_2$

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.[10] The lowest percentage of ureido esters was obtained in reactions with DEG.

Following these literature data two experiments were perfomed: 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:

$$\text{Z-NH-CH(R)-COOH} + \text{Btc-NH-CH(R)-COOR'} \xrightarrow{\text{-BtH,-CO}_2}$$
$$\textbf{4}$$

$$\text{Z-NH-CH(R)-CONH-CH(R)-COOR'}$$
$$\textbf{9a-g}$$

$$\text{Z-NH-CH(R)-CONH-CH(R)-COOH} + \textbf{4} \longrightarrow$$
$$\text{Z-NH-CH(R)-CONH-CH(R)-CONH-CH(R)-COOR'}$$
$$\textbf{10a-c}$$

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 F_{254} "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-OMe[25] and H-*L*-Phe-OBzl[26] 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-C_6H_{11}) (6a)

A suspension of 2.21 g (7 mmol) of Btc-***D,L***-Ala-NH-c-C_6H_{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): νmax 3500-2500, 3280, 1675, 1545 cm^{-1}.

D,L-Phenylglycine Butylamide (H-D,L-Pgly-NHC_4H_9) (6b)

From 2.11 g (6 mmol) Btc-***D,L***-Pgly-NHC_4H_9 (**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): νmax 3660-2400, 1665, 1555, 1475 cm^{-1}.

$C_{12}H_{19}ClN_2O$ (242.75)	*calc.*	C 59.37	H 7.88	N 11.54
	found	59.35	8.09	11.72

TABLE II

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

REACTANTS		METHOD	SOLVENT	REACTION TIME / h	PRODUCT	YIELD / %	m. p. (°C)	SOLVENT FOR RECRYST.	IR (KBr or film) (cm^{-1})
Gly-OMe·HCl	BtcCl+NMM	B	benzene	72	Btc-Gly-OMe (**4a**)	85	133-134	acetone/ water	3370, 1745, 1530
Btc-Gly-Cl	BzlOH+TEA	A	benzene	1	Btc-Gly-OBzl (**4b**)	52	150-151	acetone, methanol	3360, 1755, 1730, 1530
Gly-OBzl-HCl	BtcCl+NMM	B	dioxane	1		46			
L-Met-OEt·HCl	BtcCl+NMM	B	dioxane	1.5	Btc-***L***-Met-OEt (**4c**)	81	oil	-	3350, 1740, 1520
Btc-***L***-Phe-Cl	MeOH	A	methanol	0.75	Btc-***L***-Phe-OMe (**4d**)	99	oil	-	3370, 1725, 1500
L-Phe-OMe·HCl	BtcCl+NMM	B	dioxane	2		77			
Btc-***L***-Phe-Cl	BzlOH+TEA	A	benzene	0.75	Btc-***L***-Phe-OBzl (**4e**)	64	oil	-	3400, 1740, 1515
L-Phe-OBzl·HCl	BtcCl+NMM	B	dioxane	1.5		82			
Btc-***D***-Pgly-Cl	MeOH	A	methanol	0.5	Btc-***L***-Pgly-OMe (**4f**)	94	72-75	benzene/ petrol-ether	3410, 1740, 1500
D-Pgly-OMe·HCl	BtcCl+NMM	B	dioxane	2		87			
D-Pgly-OMe·HCl	BtcCl+DEG	B	dioxane	2		80			
Btc-***D,L***-Ala-Cl	BzlOH+TEA	A	benzene	0.25	Btc-***D,L***-Ala-OBzl (**4g**)	72	70-71	ether/ petrol-ether	3340, 1755, 1730, 1525
Btc-***L***-Leu-Cl	BzlOH+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**)

COMPOUND	REACT. TIME (h)	YIELD (%)	m. p. (°C)	Lit. m. p. (°C)	SOLVENT FOR RECRYST.	$[\alpha]_D^{20}$	Lit. $[\alpha]_D^{24}$	IR (KBr) (cm^{-1})
Z-Gly-Gly-OMe (**9a**)	6.5	60	63-65	63-65[13] 66.5-67.5[14]	ethyl acetate/ petrolether	-	-	3320, 1740, 1690, 1660, 1530
Z-Gly-Gly-OBzl (**9b**)	7.0	55	110-112	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	66	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,$CHCl_3$)	-	3300, 1725, 1690, 1660, 1530
Z-Gly-***L***-Phe-OBzl (**9f**)	13.0	68	68-69	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 (**9g**)	8.5	75	152-153	149-150[21] 156-157[20]	methanol	+10.8° (c 2, $CHCl_3$)	+9.1° (c 2, $CHCl_3$)[20]	3300, 1735, 1700, 1655, 1550
Z-***L***-Tyr(Bzl)-Gly-Gly-OMe (**10a**)	12.0	65	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	-	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, $CHCl_3$)	-	3270, 1735, 1685, 1640, 1520

* t = 23°C   ** t = 25°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}.

$C_{14}H_{20}N_2O_2$ (248.33)	*calc.*	N 11.28
	found	11.03

D,L-Phenylglycine Cyclohexylamide (H-D,L-Pgly-NH-c-C_6H_{11}) (**6c**)

0.16 g (84%) **6c** was prepared from 0.26 g (0.7 mmol) Btc-***D,L***- Pgly-NH-c-C_6H_{11} (**3e**) in 3 ml acetone and 3 ml 5% HCl. m.p. 121-124°C.

IR(KBr): νmax 3500-2500, 1655, 1570, 1510 cm^{-1}.

$C_{14}H_{21}ClN_2O$ (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-$NHCH_2C_6H_5$) (**6d**) *and 3-Benzyl-5-phenylhydantoin* (**5a**)

2.69 g (7 mmol) Btc-***D,L***-Pgly-$NHCH_2C_6H_5$) (**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): νmax 3500, 1770, 1715 cm^{-1}.

$C_{16}H_{13}N_2O_2$ (265.29)	*calc.*	C 72.44	H 4.94	N 10.56
	found	72.37	5.21	10.43

^1H NMR ($CDCl_3$) **d**(ppm): 4.67(s,2H,CH_2); 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): νmax 3330, 3290, 1645, 1515 cm^{-1}.

$C_{15}H_{16}N_2O$ (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): νmax 3400-2500, 1665, 1560, 1485 cm^{-1}.

L-Phenylalanine Benzylamide (H-L-Phe-$NHCH_2C_6H_5$) (**6e**) *and 3,5-dibenzylhydantoin* (**5c**)

a) 3.57 g (9 mmol) Btc-*L*-Phe-$NHCH_2C_6H_5$) (**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 substance[3]). 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): νmax 3500-2500, 3410, 1665, 1535 cm^{-1}.

$C_{16}H_{19}ClN_2O$ (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 Na_2SO_4 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-Me thioninol) **(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-D,L-Pgly-NHC_4H_9 (**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

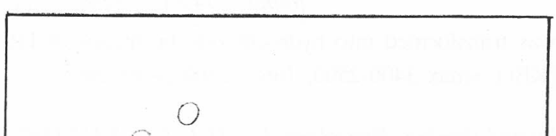


Figure 1. Acidolysis of Btc-amino acid esters: TLC of starting and final compounds

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).

$C_{16}H_{14}N_4O_3$ (310.32)	*calc.*	C 61.93	H 4.55	N 18.05
	found	61.68	4.47	18.07

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° (c 0.5, $CHCl_3$).

$C_{17}H_{16}N_4O_3$ (324.35)	*calc.*	C 62.96	H 4.97	N 17.27
	found	62.72	5.18	17.53

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° (c 2.6, $CHCl_3$).

$C_{23}H_{20}N_4O_3$ (400.44)	*calc.*	C 68.99	H 5.03	N 13.99
	found	69.23	5.23	14.11

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, $CHCl_3$).

$C_{16}H_{14}N_4O_3$ (310.31)	*calc.*	C 61.93	H 4.55	N 18.06
	found	61.74	5.08	18.06

*N-Btc-**D,L**-Alanine benzyl ester (Btc-D,L-Ala-OBzl)* (**4g**): The crude product was recrystallized from ether/petroleum ether.

$C_{17}H_{16}N_4O_3$ (324.35)	*calc.*	C 62.96	H 4.97	N 17.27
	found	62.73	5.26	17.29

^{1}H NMR ($CDCl_3$) *d*(ppm): 1.65(d,3H,CH_3); 4.86(m,1H,CH); 5.25(s,2H,CH_2); 7.84(m,9$H_{arom.}$ 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, $CHCl_3$).

$C_{20}H_{22}N_4O_3$ (366.42)	*calc.*	C 65.56	H 6.05	N 15.29
	found	65.76	6.12	15.20

^{1}H NMR ($CDCl_3$) *d*(ppm): 0.99(d,6H,2CH_3); 1.83(m,3H,$CHCH_2CHCO$); 4.80(m,1H,CHN); 5.23(s,2H,CH_2O); 7.82(m,9$H_{arom.}$ 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 suspension, 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.

$C_{10}H_{10}N_4O_3$ (234.22)	*calc.*	C 51.28	H 4.30	N 23.92
	found	50.99	4.58	23.82

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, $CHCl_3$).

$C_{14}H_{18}N_4O_3S$ (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): νmax 3350, 1740, 1635, 1575 cm^{-1}.

N-Btc-L-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): νmax 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): νmax 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-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.

$C_{24}H_{30}N_2O_5S$ (458.57)	*calc.*	C	62.86	H	6.59	N	6.11
	found		62.95		6.75		6.37

^{1}H NMR ($CDCl_3$) *d*(ppm): 1.26(t,3H,CH_3CH_2); 1,78(m,2H,CH_2CH_2S); 2.04(s,3H,CH_3S); 2.29(m,2H,CH_2S); 3.08(d,2H,CH_2CH); 4.17(q,2H,CH_3CH_2O); 4.55(m,2H,2CHCO); 5.09 (s,2H,CH_2O); 5.27(d,1H,CONH); 6.46(d,1H,NHCOO); 7.28(m,10$H_{arom.}$).

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.

$C_{26}H_{33}N_3O_6S$ (515.63)	*calc.*	C	60.56	H	6.45	N	8.15
	found		60.83		6.65		7.96

^{1}H NMR ($CDCl_3$) *d*(ppm): 1.54(t,3H,CH_3); 2,02(s,3H,CH_3S); 2.27(m,4H,CH_2CH_2S); 3.04(d,2H,CH_2CHCO); 3.83(d,2H,CH_2CO); 4.13(q,2H,OCH_2CH_3); 4.65(m,2H,CHCO); 5.09 (s,2H,CH_2O); 5.67(m,1H,NH); 6.93(m,2H,2NH); 7.20(m,10$H_{arom.}$).

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**.

$C_{26}H_{33}N_3O_6$ (483.57)	*calc.*	C	64.58	H	6.88	N	8.69
	found		64.86		6.95		8.74

REFERENCES

1. I. Butula, B. Zorc, and V. Vela, *Croat. Chem. Acta* **54** (1981) 435.
2. I. Butula, B. Zorc, M. Ljubić, and G. Karlović, *Synthesis* (1983) 327.
3. B. Zorc and I. Butula, *Croat. Chem. Acta* **54** (1981) 441.
4. I. Butula, M. V. Proštenik, and V. Vela, *Croat. Chem. Acta* **49** (1977) 837.
5. T. Wieland, *Acta Chim. Acad. Sci. Hung.* **44** (1965) 5.
6. H. A. Staab and W. Benz, *Ann. Chem.* **648** (1961) 72, 82.
7. S. Goldschmidt and M. Wick, *Ann. Chem.* **575** (1952) 217.
8. J. Gante, *Angew. Chem.* **78** (1966) 334, 602.
9. J. Gante, *Chem. Ber.* **99** (1966) 2521.
10. S. Sakakibara and M. Itoh, *Bull. Chem. Soc.* **40** (1967) 646.
11. Houben-Weyl, *Methoden der organischen Chemie,* Vierte Auflage, Herausgegeben von Eugen Müler, *Synthese von Peptiden I u. II.* (Vol. **15/1** u. **15/2**), Georg Thieme Verlag, Stuttgart, 1974.
12. E. Schröder and K. Lubke, *The Peptides,* Vol. **1** and **2**, Academic Press, Inc., New York and London, 1965.
13. N. F. Albertson and F. C. McKay, *J. Am. Chem. Soc.* **75** (1953) 5323
14. Y. A. Bara, A. Friedrich, W. Hehlein, H. Kessler, P. Kondor, M. Molter, and H. J. Veith, *Chem. Ber.* **111** (1978) 1029.
15. D. B. Ishai, *J. Org. Chem.* **19** (1954) 62.
16. L. Zervas and D. Theodoropoulos, *J. Am. Chem. Soc.* **78** (1956) 1359.
17. D. Theodoropoulos and J. Gazopoulos, *J. Org. Chem.* **27** (1962) 2091.
18. J. R. Vaughan and J. A. Eichler, *J. Am. Chem. Soc.* **75** (1953) 5556.
19. J. S. Morley, *J. Chem. Soc. (C)* (1967) 2410.
20. M. Fujino and O. Nishimura, *Chem. Pharm. Bull.* **17** (1969) 1937.
21. S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Japan.* **38** (1965) 1979.
22. H. Yajima, N. Mizokami, M. Kiso, T. Jinnouchi, Y. Kai and Y. Kiso, *Chem. Pharm. Bull.* **22** (1974) 1075.
23. P. Ruglli, R. Ratti, and E. Henzi, *Helv. Him. Acta* **12** (1929) 332.
24. D. Fleš and A. Markovac-Prpić, *Croat. Chem. Acta* **29** (1957) 79.
25. H. Reihlen and L. Knöpfle, *Ann. Chem.* **523** (1936) 199.
26. J. Matijević-Sosa, B. Zorc, and I. Butula, *Croat. Chem. Acta,* **58** (1985) 239.
27. E. Wünsch, G. Fries, and A. Zwick, *Chem. Ber.* **91** (1958) 542.
28. H. R. Krickeldorf and G. Greber, *Chem. Ber.* **104** (1971) 3168.
29. W. Bauer, F. Cardinaux, R. Huguenin, and J. Pless, DOS 2,702,711 (1977), Sandoz; C. A. **87**, 184970 (1977).
30. K. Kondo, K. Murata, N. Miyoshi, S. Murai, and N. Sonoda, *Synthesis,* (1979) 735.

SAŽETAK

Reakcije s *N*-(1-benzotriazolilkarbonil)-aminokiselinama.
IV. Upotreba derivata *N*-(1-benzotriazolilkarbonil)-aminokiselina u sintezi peptida

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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 CO_2, odgovarajuće terminalno zaštićene di- i tripeptide. Diskutira se o nedostacima i prednostima ovih reakcija.