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THE SYNTHESIS OF DIPEPTIDES WHICH CONTAIN α-AMINONITRILES AS THEIR C-TERMINAL RESIDUES

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The synthesis of some dipeptides which contain α-aminonitriles as their C-terminal residues is described. The coupling reactions of active esters of N-tritylamino acids, p-nitrophenyl esters or cyanomethyl esters, with α-aminonitriles were carried out in N,N-dimethylformamide. The N-trityl protecting groups of the reaction products were removed by acid hydrolysis without affecting their nitrile groups. In this way, glycylaminoacetonitrile acetate(8), glycyl-DL-α-aminopropionitrile acetate(10) and DL-alanylaminoacetonitrile acetate(11) were synthesized.

Liberek et al.[1] reported the synthesis of oligopeptides containing nitrile groups in their side chains, e.g. a cyano-L-alanyl peptide, an analog of the C-terminal tetrapeptide amide sequence of Gastrin, but there has been no report on a peptide the C-terminal residue of which is an α-aminonitrile. The coupling reactions of active esters of N-tritylamino acids with α-aminonitriles were investigated to get such peptides as follows.

$$\underset{\text{(I)}}{T\text{-}NH\text{-}CH(R_1)\text{-}CO\text{-}X} + \underset{\text{(II)}}{H_2N\text{-}CH(R_2)CN} \longrightarrow \underset{\text{(III)}}{T\text{-}NH\text{-}CH(R_1)\text{-}CO\text{-}NH\text{-}CH(R_2)\text{-}CN}$$

(I);	R_1= H	X= O--NO_2	(1)
	CH_3	O--NO_2	(2)
	H	O-CH_2-CN	(3)
	CH_3	O-CH_2-CN	(4)
(II);	R_2= H	aminoacetonitrile	
	CH_3	DL-α-aminopropionitrile	
(III);	R_1= H	R_2= H	(5)
	H	CH_3	(6)
	CH_3	H	(7)

T; Trityl group

As a N-protecting group, the trityl group was introduced because it was easily cleaved by aqueous acetic acid or trifluoroacetic acid without hydration of the nitrile group. N-Tritylglycine and N-trityl-DL-alanine were prepared according to Zervas' pro-

cedure.[2] Since the alkaline hydrolysis of N-trityl-DL-alanine methyl ester according to this procedure yielded no product, the hydrolysis condition was slightly modified with raising a reaction temperature to 50°C. The N-tritylamino acids obtained were converted to the active esters by the action of p-nitrophenol and N,N'-dicyclohexylcarbodiimide in ethyl acetate. Cyanomethyl active esters were prepared according to Schwyzer's procedure.[3] These active esters are shown in Table 1.

Table 1. The Synthesis of the Active Esters

Active Esters		Formula	M.p.(°C)	Yield(%)
N-Tritylglycine p-nitrophenyl ester	(1)	$C_{27}H_{22}O_4N_2$	147-150[a]	31
N-Trityl-DL-alanine p-nitrophenyl ester	(2)	$C_{28}H_{24}O_4N_2$	110-112	95
N-Tritylglycine cyanomethyl ester	(3)	$C_{23}H_{20}O_2N_2$	89-90[b]	90
N-Trityl-DL-alanine cyanomethyl ester	(4)	$C_{24}H_{22}O_2N_2$	173-175	70

a) reported value: 153°C(Ref. 4) b) reported value: 90°C(Ref 3)

These active esters were coupled with α-aminonitriles, aminoacetonitrile or DL-α-aminopropionitrile. The solution of a free α-aminonitrile in N,N-dimethylformamide(DMF) was prepared by adding a slight excess of triethylamine to the α-aminonitrile sulfate suspended in DMF. There was a difference in the coupling reactivity between the p-nitrophenyl esters and the cyanomethyl esters.

The coupling reaction of the p-nitrophenyl ester with the α-aminonitrile, that of N-tritylglycine p-nitrophenyl ester(1) with aminoacetonitrile, that of (1) with DL-α-aminopropionitrile or that of N-trityl-DL-alanine p-nitrophenyl ester(2) with aminoacetonitrile yielded the product, N-tritylglycylaminoacetonitrile(5), N-tritylglycyl-DL-α-aminopropionitrile(6) or N-trityl-DL-alanylaminoacetonitrile(7) respectively. However, N-trityl-DL-alanyl-DL-α-aminopropionitrile was not formed by means of this procedure.

For example: N-Tritylglycine p-nitrophenyl ester(1) was added to the solution containing free aminoacetonitrile and stood for 24 hours at room temperature. After addition of a large excess of water to the reaction mixture, N-tritylglycylaminoacetonitrile(5) was precipitated, which was recrystallized from ethanol.

On the other hand, the coupling reactions of the cyanomethyl esters with the α-aminonitriles yielded only (5) and no other.

For example: N-Tritylglycine cyanomethyl ester(3) and a few drops of glacial acetic acid were added to the solution containing free aminoacetonitrile, the resulting solution was heated for 40 minutes at 60°C and stood for 12 hours at room temperature. After addition of a large excess of water, (5) was precipitated.

Table 2. The Synthesis of the N-Trityldipeptides Containing the α-Aminonitriles

N-Trityldipeptide		Formula	M.p.(°C)	Yield(%)
N-Tritylglycylaminoacetonitrile	(5)	$C_{23}H_{21}ON_3$	203-205	70
			205	80[c]
N-Tritylglycyl-DL-α-aminopropionitrile	(6)	$C_{24}H_{23}ON_3$	179-180	46
N-Trityl-DL-alanylaminoacetonitrile	(7)	$C_{24}H_{23}ON_3$	190-192	50

c) obtained from cyanomethyl ester

The obtained N-trityldipeptides containing the α-aminonitriles as their C-terminal residues are shown in Table 2. The yield of the N-trityldipeptide was lower when one partner of a couple was an alanyl derivative as an active ester or as an α-aminonitrile. This could be attributed to the steric hindrance of the bulky trityl group.

N-Trityl-DL-alanyl-DL-α-aminopropionitrile was not formed even at the elevated reaction temperature of 60°C. Although Zervas et al.[4] previously pointed out that N-tritylamino acid p-nitrophenyl esters except the glycyl derivative were not coupled with amino acid esters or peptide esters because of steric hindrance, N-trityl-DL-alanyl-DL-alanine ethyl ester was formed by the coupling reaction at the temperature of 60°C (M.p. 195-198°C, yield 72%). The poor reactivity of DL-α-aminopropionitrile may be due to the low electron density on its amino nitrogen atom.

Two kinds of acids, aqueous acetic acid(50%) and trifluoroacetic acid, were used to remove the N-trityl group.

For example(using aqueous acetic acid): N-Tritylglycylaminoacetonitrile(5) suspended in aqueous acetic acid was heated for 5 minutes at 95°C to be dissolved and triphenylcarbinol was precipitated simultaneously. After water had been added to complete the precipitation of the carbinol, the precipitates were removed by filtration. The filtrate was concentrated to a smaller volume, and mixed with ethanol. The resulting mixture was allowed to stand overnight in a refrigerator to make glycylaminoacetonitrile acetate(8) crystallize.

For example(using trifluoroacetic acid): N-Tritylglycylaminoacetonitrile(5) was dissolved in anhydrous trifluoroacetic acid. On cooling below the temperature of -5°C, a smaller volume of water was added gradually to the yellowish brown solution. Triphenylcarbinol precipitated was removed by filtration, and then the filtrate was concentrated under reduced pressure. After ethanol and a larger volume of ether had been added to the concentrated solution, glycylaminoacetonitrile trifluoroacetate(9) was precipitated.

The detritylation products obtained are shown in Table 3.

Table 3. The Detritylation of the N-Trityldipeptide Derivatives

Detritylated peptide		Formula	M.p.(°C)	Yield(%)
Glycylaminoacetonitrile acetate	(8)	$C_6H_{11}O_3N_3$	133-135	75
Glycylaminoacetonitrile trifluoroacetate	(9)	$C_6H_8O_3F_3N_3$	112	74
Glycyl-DL-α-aminopropionitrile acetate	(10)	$C_7H_{13}O_3N_3$	120-122	85
DL-Alanylaminoacetonitrile acetate	(11)	$C_7H_{13}O_3N_3$	106-108	92
DL-Alanylaminoacetonitrile trifluoroacetate	(12)	$C_7H_{10}O_3F_3N_3$	129-131	42

When these peptides were analyzed by use of an amino acid analyzer, only one peak except a small peak due to ammonia was observed each after that of ammonia. Therefore, these samples were chromatographycally pure. When they were analyzed after being hydrolyzed completely in boiling 6N-HCl, the peaks due to the corresponding amino acids and ammonia were detected exclusively in their chromatograms.

For example: Glycylaminoacetonitrile acetate(8) (0.560 mmol) was hydrolyzed and then glycine(1.14 mmol) and ammonia(0.637 mmol) were recovered. These values agree

with the amino acid composition of (8).

The paper chromatography of these peptides developed with n-butanol–acetic acid–water system(4:1:2 v/v) gave the spots, the colors and R_f values of which are shown in Table 4. Any extra spot due to an impurity was not observed in their chromatograms.

Table 4. The Results of Paper Chromatography

Peptide		R_f	Color[d)]
Glycylaminoacetonitrile acetate	(8)	0.21	yellow
Glycyl-DL-α-aminopropionitrile acetate	(10)	0.30	yellow
DL-Alanylaminoacetonitrile acetate	(11)	0.28	violet
DL-α-Aminopropionitrile trifluoroacetate		0.60	violet

d) ninhydrin color

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