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THERMAL SYNTHESIS OF THE OPTICAL PURE PENTAPEPTIDE DERIVATIVE Z-(L)-Ala-(L)-Phe-Gly-(L)-Phe-Gly-OMe

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Summary:

The pentapeptide Z-(L)-Ala-(L)-Phe-Gly-(L)-Phe-Gly-OMe was obtained by reacting the activated dipeptide derivative Z-(L)-Ala-(L)-Phe-OPcp with the amino peptide derivative TFA·H-Gly-(L)--Phe-Gly-OMe at 100-105°C under reduced pressure (10^{-2}-10^{-3} Torr) without using solvents. The product obtained by bulk condensation showed no racemization, whereas the product obtained by a matrix mediated condensation contained 5,5 % diastereomer Z-(L)-Ala-(D)-Phe-Gly-(L)-Phe--Gly-OMe. Separation of diastereomers was achieved by HPLC on a silicagel column.

According to Goodman et al.[1,2], activated tri- and tetra-peptide esters which are adsorbed on a diatomaeous earth matrix, polymerize without racemization to polypeptides when heated at temperatures of 100-105°C in high vacuum.

It has been our intention to apply this principle of synthesis to the contruction of defined peptides. One of several model peptides which we have synthesized by this method, and which meets the numerous critèria required for this study has the sequence:

Z-(L)-Ala-(L)-Phe-Gly-(L)-Phe-Gly-OMe.

We chose benzyloxycarbonyl-(L)-alanine-(L)-phenylalanine-pentachlorophenylester (Z-(L)-Ala--(L)-Phe-OPcp) and the trifluoroacetate salt of glycine-(L)-phenylalanine-glycine-methylester (TFA·H-Gly-(L)-Phe-Gly-OMe) as starting compounds for the synthesis.

The reaction followed equation I:

Equation I: Z-(L)-Ala-(L)-Phe-OPcp + TFA · H-Gly-(L)-Phe-Gly-OMe→
Z-(L)-Ala-(L)-Phe-Gly-(L)-Phe-Gly-OMe + HOPcp + TFA

The starting compounds were synthesized by the mixed anhydride method. To ensure that the

dipeptide pentachlorophenyl ester was free of racemization, we employed the "backup route"[7,2] by coupling HBr • H-(L)-Phe-OPcp with Z-(L)-Ala-OH via the mixed anhydride method. The ester was found to be free of racemization by ^{1}H-NMR-spectroscopy[4,5]. The diastereomeric pentachlorophenyl peptide esters have different chemical shifts for the methyl doublets of alanine (1.0 ppm - 1.1 ppm).

The first attempt at coupling the dipeptide ester with the trifluoroacetate salt of the tripeptide was performed on a diatomaceous earth matrix (Hyflo Super Cel^{R}) at a temperature of 100 - 105°C in a vacuum of 10^{-2} - 10^{-3} Torr. After 47 hours only incomplete reaction was obtained. The product C was separated from the matrix by chloroform extraction.

A parallel reaction, a bulk condensation under the same reaction conditions, led within 83 hours to product D. In both cases, the degree of reaction was controlled by TLC. Counter current distribution in a toluene systeme[8)] afforded the products in high purity. The yield was 20 % for the matrix-mediated, and 18 % for the bulk condensation product.

To establish the degree of racemization occuring during the thermal coupling, we synthesized by conventional methods the diastereomeric pentapeptides A and B as references. This permitted us to compare their characteristics with these of the thermal products C and D.

A) Z-(L)-Ala-(L)-Phe-Gly-(L)-Phe-Gly-OMe
B) Z-(L)-Ala-(D)-Phe-Gly-(L)-Phe-Gly-OMe.

The amount of diastereomer in the pentapeptides C and D, was monitored by optical rotation measurements, and by enzymatic digestion of acid hydrolysate with D-amino acid oxidase[3,9,10]. The diastereomeric components could also be separated by HPLC, allowing determination of the ratio of the diastereomers by comparison with the standard peptides A and B. (For results see scheme I)

In the evaluation of the enzymatically-determined degree of racemization one must take into account that free L-Phe is partially converted into D-Phe (ca. 2 %) during the acid hydrolysis step[9]. Phenylalanine within peptides may be racemized up to 45 % during the hydrolysis, depending on the neighbouring amino acids[9]! This method, although rapid, can only be used as a rough screening method which allows no useful determinations of low (5 %) degrees of racemization.

The HPLC separation was a baseline separation and was performed on a Perkin Elmer (Series 3) instrument. The column was from Perkin-Elmer, (Silica A, 10 µm particle size). The eluant was a mixture of chloroform and methanol (100:1,5).

The error in the determination of the D-diastereomer content was reckoned to be 0,3 %, so that lower degrees of racemization than 5 % can be reasonably well determined.

Scheme I:

Samples:	Analytical data	% Diastereomer, determined by: D-amino acid oxidase	HPLC
compound A:			
amino acid analysis	Ala(1):Phe(2):Gly(2)		
found:	0,97 1,90 2,00		
α_D^{22} :	-19,6° ± 0,5 c=1 DMF	8	0,0 ± 0,3
mp:	196 - 197°C		
compound B:			
amino acid analysis	Ala(1):Phe(2):Gly(2)		
found:	1,08 1,98 2,00		
α_D^{22} :	-6,1° ± 0,5 c=1 DMF	104	100,0 ± 0,3
mp :	175 - 179°C		
compound C:			
amino acid analysis	Ala(1):Phe(2):Gly(2)		
found:	1,00 1,99 2,00		
α_D^{22} :	-19,1° ± 0,5 c=1 DMF	-4	5,5 ± 0,3
mp :	190 - 195°C		
compound D: (bulk condensation)			
amino acid analysis	Ala(1):Phe(2):Gly(2)		
found:	0,99 2,02 2,00		
α_D^{22} :	-19,9 ± 0,5 c=1 DMF	-2	0,0 ± 0,3

Sample:	Analytical data	method of identification of racemization:	^{1}H-NMR-spectroscopy
Z-(L)-Ala-(L)-Phe-OPcp			
amino acid analysis	Ala(1):Phe(1)		
found:	1,00 1,00		no racemization
α_D^{22} :	-67,5° ± 0,5 c=1 DMF		

In summary, it is feasible to use thermal condensation of peptide derivatives for the synthesis of polypeptides of defined sequence.The use of a matrix, however, leads to increased racemization (11 %) in comparison to that found in bulk condensations where virtually no racemization can be observed.

The low yields of 18 to 20 % could be accounted for side reactions, for example homopolymerisation[11] of the amino component or reduced movement of the reacting components. Further investigations are under way to understand the role of side reactions in order to improve the yield of the coupling.

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