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FRAGMENTS COUPLING IN PEPTIDE SYNTHESIS 1.
THE BASIS OF QUANTITATIVE RELATIONSHIPS BETWEEN STRUCTURE AND EPIMERIZATION

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The epimerization (racemization) of the activated aminoacid during fragments coupling in peptide synthesis has been extensively studied concerning the effects of the coupling method, the solvent, the base and the ions present in the solution, through the racemization tests [1].

However, almost nothing is available concerning the influence of the structure of the fragments to be condensed [2]. It would be of the highest interest to the chemist undertaking a long peptide synthesis to dispose of predictive indexes indicating which fragments are to be planned to be coupled, minimizing the risk of epimerization. We give here the basis of our approach to this problem and some preliminary results.

Our method is based on the following points :

1 - We assume that the coupling of two fragments F_1-AiAjOH and H_2NAk-F_2 depends mainly on the nature of the three aminoacids Ai, participating aminoacid in the oxazolone formation, Aj, activated aminoacid, and Ak, the amino component. Hence the coupling of Boc AiAjOH on H_2NAkOMe is a suitable model reaction.

2 - An epimerization test is needed. We have found that the ratio of the diastereoisomeric pairs Boc L-Ai-L-Aj-L-Ak OMe and Boc L-Ai-D-Aj-L-Ak OMe can be measured by the integration of the 250 MHz ^{1}H NMR signals of the methylesters ; the signals of most of 20 triplets examined were generally separated by better than 3 Hz (generally 7-10 Hz) (see table I and II). This test fails in the only case of a C-terminal phenylalanine where the separation is under 2 Hz [4]. This test is actually an extension of the Davies'observations [5].

3 - A kinetic treatment, based on a factorization of the rate constants used by KEMP [6] for the rates of coupling, is here applied to the competition "coupling versus epimerization". It gives a predictive equation for the epimerization ratio :

$$\rho_{ijk} = \frac{LDL}{LLL} = \frac{\rho_{ioo} \times \rho_{ojo} \times \rho_{ook}}{\rho^2_{ooo}}$$

where the index o refers to a standard aminoacid in each position (we have choosen alanin).

This equation is valid if all the measurements are performed in the same conditions : coupling method, solvent, base and concentration [7].

If this equation is experimentally valid for a significant population of tripeptides, it could provide a prediction for the epimerization of every triplet configuration ijk. If we exclude the straight forward cases of glycin and prolin and consider 18 aminoacids, the measurements for (3x17) + 1 = 52 references will give an access to the prediction of $(18)^3$ = 5832 cases ! This would be a powerfull aid for fixing a strategy of a fragments synthesis.

We report now our results on the five non functionnal aminoacids needing 13 references for the prediction of 125 triplets. For each measurement, both references Boc L-Ai-L-Aj-L-Ak OMe and Boc L-Ai-D-Aj-L-Ak OMe are synthetized stepwise using our BOP reagent for the coupling [3]. Then the dipeptide Boc L-Ai-L-Aj OH is coupled in DMF at the concentration of 10^{-1} M with the stoechiometric amount of Cl^-, H_3N^+-L-Ak OMe, with BOP and NEt_3 (stoechiometric amounts). NMR measurements are made in standardized conditions.

Boc L-Ai-L-Aj OH is obtained by saponification of its methylester. As epimerization can be suspected in this reaction [8], we have tested on the case of Boc-Val-Val-OH that the reesterification with diazomethane affords a product with unchanged characteristics with respect to the starting ester.

The table I gives the values of the references ; six random triplets are reported in table II, showing an excellent fit.

Phenylalanin is seen to have a striking favorable effect in every position. This observation is an a posteriori justification of the systematic use of phenylalanin activation in some synthesis [9].

The fit is an encouraging indication though it is not statistically fully significant. In a further paper, we shall give the complete kinetic treatment leading to our equation, an experimental verification based on statistical methods, and the error treatment.

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Table I

Tripeptides Boc-AiAjAkOMe	ν_{LLL} (Hz)	ν_{LDL} (a) (Hz)	$\Delta\nu$ (Hz)	$\rho ijk = \frac{LDL}{LLL}$ %
Ala-Ala-Ala	936	930	6	6,7
Ala-Ala-Phe	923.5	922	1.5	1
Ala-Ala-Val	937.5	930	7.5	9.3
Ala-Ala-Leu	932	923	9	5.7
Ala-Ala-Ile	934.5	928	6.5	6.5
Phe-Ala-Ala	933.5	924.5	9	5.4
Val-Ala-Ala	936	930	6	8.7
Leu-Ala-Ala	935.5	930	5.5	6.1
Ile-Ala-Ala	936	931	5	8.1
Ala-Phe-Ala	925	920	5	$\sim$ 0.1
Ala-Val-Ala	934.5	930	4.5	17.2
Ala-Leu-Ala	933.5	928.5	5	1.5
Ala-Ile-Ala	933	930	3	19

(a) ν : observed frequencies for the methylester singlet in ^1H NMR at 250 MHz (CAMECA RMN 250)

Table II

Tripeptides Boc-AiAjAkOMe	ν_{LLL}	ν_{LDL}	ρ calc. %	ρ measured %
Phe-Ala-Val	936	926	7.4	7.5
Phe-Ala-Leu	930.5	921.5	4.5	4.5
Phe-Ala-Ile	933	924	5.3	5.3
Val-Ile-Val	932.5	925	34	32.5
Leu-Val-Ile	932	926	15	11.4
Val-Val-Leu	928.5	920	18.9	17.2

References and notes

[1] M. Bodanszky, Y.S. Klausner, M.A. Ondetti, Peptide Synthesis, 2d Ed. Wiley and Sons Ed. 1976, 146, 153

[2] See ref. (1) p. 148

[3] B. Castro, J.R. Dormoy, B. Dourtoglou, G. Evin, C. Selve, J.C. Ziegler, Synthesis, 1976, 11, 751

[4] Other NMR tests are in progress overcoming this limitation.

[5] J.S. Davies, M.N. Ibrahim, Tetrahedron Letters, 1977, 17, 1453

Nevertheless, it is clear from our results that the presence of an aromatic ring is not necessary for the separation of the signals.

[6] P.S. Kemp, S.L.H. Choong, J. Pekaak, J. Org. Chem. 1974, 39, 3841

[7] This point is peculiarly important and misregarded by most authors using racemization tests ; actually it can be seen that the racemization increases with the inverse of the concentration.

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