Tetrahedron Letters No. 6, pp 447 - 448, 1976. Pergamon Press. Printed in Great Britain.

# A SIMPLIFIED METHOD FOR THE DETERMINATION OF RACEMIZATION AND BY-PRODUCT FORMATION IN PEPTIDE SYNTHESIS

Anatol ARENDT, Aleksander KOŁODZIEJCZYK, Teresa SOKOŁOWSKA*
Department of Organic Chemistry, Technical University, 80-952 Gdańsk, POLAND

(Received in UK 22 December 1975; accepted for publication 24 December 1975)

The coupling process and other stages of peptide synthesis are often accompanied by reactions giving rise to undesirable by-products of modified configuration or structure. Quantitative determination of these products is indispensable for evaluating and improving the methods to be used in synthesis.

Up to the present only a few racemization tests have been performed by quantitative determination of the racemates or diastereoisomers which form as by-products in model syntheses [1]. Racemization tests usually require laborious preliminary procedures, such as crystallization, deprotection, hydrolysis and/or suitable derivatization, which are all likely to affect the ratios of the stereoisomers originally formed. It has, until now, not been possible to increase the number of simple model peptides, because of difficulties which have been experienced in the separation of diastereoisomers of protected peptides. TLC separation of these diastereisomers has so far been reported only in a few cases[2], each of which was considered an event.

Our methodical search for suitable conditions for the separation of diastereoisomers of protected dipeptides by TLC has proven successful. Complete separation on thin layers of silica gel was achieved for about 50 of the 80 models investigated, containing a variety of amino acids and protecting groups[3].

Quantitative determination of microgram amounts of protected dipeptides separated by TLC was performed by liquid scintillation counting, basing the procedure on tritium radioactivity. Tritium can be introduced into the amino acid side chain or the protecting group of a substrate used in the synthesis.

An illustration of how the method proposed here is utilized in the study of racemization, is the determination of the extent of epimerization in variously modified syntheses performed by the DCC method. N-formyl-phenylalanyl-phenylalanine methyl ester was synthesized from optically pure N-formyl-D.phenylalanine which was tritium-labelled in the aromatic ring (specific activity 6 mCi/mmole) and L.phenylalanine methyl ester hydrochloride, in the presence of a tertiary amine and some racemization-suppressing additives [4].
The syntheses were performed on a 0.01 mmole scale. The crude reaction mixtures were spotted on TLC plates and developed in solvent systems containing diisopropyl ether and isopropanol (10:1). Three resolved spots were detected in

UV light.The relative amounts of the three radioactive substances were determinated: these compounds were the expected product CHO.D.Phe-L.Phe.OMe, the epimeric by-product CHO.L.Phe-L.Phe.OMe,and the acyl urea derivative N(CHO.D.Phe)-dicyclohexylurea,which is a by-product of the DCC based synthesis [5]. For the detrmination,the solid support was scraped off a 1 cm x 1 cm area including the whole spot,and transferred to measuring vials.The total activity was measured using toluene scintillating solution containg 4 g of PPO, 0.2 g of POPOP and 50 ccm of ethanol per liter.Results are shown in the Table.

Table: Epimerization degree A and acyl urea formation B during DCC coupling
Reaction: CHO.D.Phe + L.Phe.OMe x HCl + Base /in DMF/

| Base | $NEt_3$ | | | | N-Me-morpholine | | | |
|---|---|---|---|---|---|---|---|---|
| Temp.°C | 30 | | 0 | | 30 | | 0 | |
| Additives | A | B | A | B | A | B | A | B |
| None | 7.3 | 61.7 | 3.7 | 38.0 | 20.2 | 53.4 | 7.5 | 31.7 |
| 2-hydroxypyridine | 8.9 | 58.1 | 4.6 | 49.4 | 17.3 | 57.4 | 9.3 | 37.4 |
| 8-hydroxyquinoline | 9.7 | 62.0 | 4.6 | 51.4 | 22.5 | 52.5 | 8.5 | 30.66 |
| p-nitrophenol | 9.7 | 61.7 | 5.9 | 50.4 | 19.8 | 51.7 | 10.7 | 33.0 |
| N-hydroxyphtalimide | 2.4 | 2.39 | 1.3 | 1.13 | 2.9 | 3.02 | 2.3 | 1.29 |
| N-hydroxysuccinimide | 1.3 | 1.77 | 1.2 | 1.26 | 1.3 | 2.22 | 1.4 | 1.50 |
| N-hydroxybenzotriazole | 3.6 | 0.20 | 2.5 | 0.21 | 2.6 | 0.49 | 2.1 | 0.16 |

Epimerization degree $A = \frac{L\text{-}L}{D\text{-}L + L\text{-}L} \cdot 100\%$ ; Acyl urea formation $B = \frac{\text{acyl urea}}{D\text{-}L + L\text{-}L + \text{acyl urea}} \cdot 100\%$
Standard deviation : $\pm 0.008$ for values ca 0.2% ; $\pm 0.03$ for values 3.6-96.0%

Of special interest in the series of results listed in the Table under A are the data on the six additives whose use is recommended. Racemization was found to be effectively prevented only by the hydroxylamine derivatives N-hydroxysuccinimide, N-hydroxyphthalimide and N-hydroxybenzotriazole.

The results of series B show that acyl urea can under certain conditions be the main product of DCC reactions involving N-formyl-phenylalanine,and that this side reaction can be markedly limited by addition of an N-hydroxy compound, in particular, of N-hydroxybenzotriazole.

The model synthesis here presented is one of the 50 we have carried out with different pairs of amino acids, or different protecting groups. Significant improvements in peptide syntheses may result from the inspection of a greater number of model reactions.

REFERENCES: 1 H.D.Jakubke, H.Jeschkeit, Aminosäuren, Peptide, Proteine, Berlin 1973, p.185
2 Z.Pravda, K.Poduška, K.Blaha, Collect.Czech.Chem.Commun. 29,2626 /1964/
P.Hubert, E.Dellacherie, J.Chromatog., 80,144/1973/
3 A.Arendt, A.Kołodziejczyk,T.Sokołowska, Chromatographia in press
4 J.E.Zimmerman, G.W.Anderson, J.Amer.Chem.Soc.,89,7175/1967/
W.König,R.Geiger, Chem.Ber.,103,778, 2024 /1970/
5 H.H.Khorana, Chem. Ind.,1955,1087
M.Löw,L.Kisfaludy, Acta Chim.Acad.Hung.,44,61 /1965/
M.Gross, L.Bilk, Tetrahedron 24,6935 /1968/