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THE CYCLISATION OF N-PROTECTED PEPTIDES

H.Faulstich, H.Trischmann und Th.Wieland

Max-Planck-Institut für medizinische Forschung,

Abteilung Chemie, Heidelberg, DBR.

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Recently H.Faulstich [1] described the synthesis of peptides by reacting thiophenylesters of N-acylated aminoacids with NPS-protected aminoacids or peptides under catalysis of imidazole, e.g.:

$$ZNH\text{-}CHR\text{-}COSC_6H_4(p\text{-}NO_2,H,CH_3) + NPS\text{-}(NH\text{-}CHR'\text{-}CO)_n\text{-}OCH_3$$

$$\xrightarrow[45^{\circ},\ 1o - 45\ Min.]{4\ eq.\ imidazole}$$

$$ZNH\text{-}CHR\text{-}CO\text{-}(NH\text{-}CHR'\text{-}CO)_n\text{-}OCH_3 + NPS\text{-}SC_6H_4(p\text{-}NO_2,H,CH_3)$$

$R,R' = CH_3, CH_2C_6H_5, CH_2SCH_2C_6H_5$ a.o.

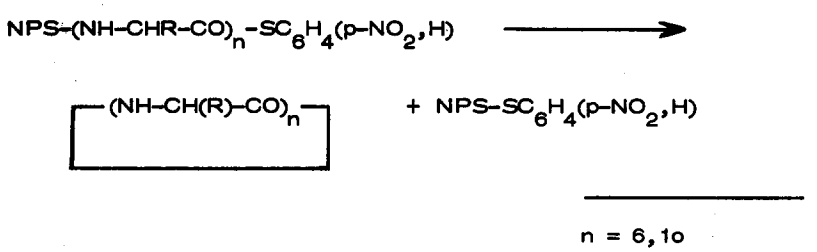
$NPS = o\text{-}NO_2C_6H_4S$

$n = 1 - 3$

Yields were as high as 9o %, the racemisation according to Anderson's test was 4 % for the p-nitrothiophenylester.

Now this procedure is found useful for the cyclisation of peptides. The cyclisation reaction was carried out in one step starting from the N-protected and carboxylactivated

peptide:



thus avoiding the rough conditions of acidolysis of the N-protecting residues with HBr/ acetic acid or trifluoracetic acid and also the deprotonation of the amino end.

The p-nitro thiophenylesters of NPS-peptides were favoured because of the low degree of racemisation found previously in the synthesis of linear peptides [1]. These esters could be purified by washing their solutions in ethylacetate with aqueous 1 N HCl and 5 % $KHCO_3$ or by tlc without decomposition or head to tail reaction. Cyclizsation did not start until imidazole and/or p-nitrothiophenol was added to the dilute solution. The latter must be added in any case, as the cleavage of the NPS-group depends on p-nitrothiophenol which is not sufficiently generated from the activated peptide end in the cyclisation reaction. To protect from oxydation the cyclisations were carried out under nitrogen. Good results were obtained in anhydrous tetrahydrofuran or in anhydrous pyridine but not in dimethylformamide, which led to side reactions with one of the components.

Using only L-aminoacids two cyclohexapeptides and one cyclodecapeptide were synthesized in the following manner:

1). NPS-valyl-alanyl-phenylalanyl-alanyl-diglycylthiophenylester was prepared by condensation of the NPS-protected tripeptide with the corresponding tripeptidethiophenylester-trifluoracetate via mixed anhydrides. The cyclisation in pyridine [o,1 mM,

3o ml anhydrous pyridine, 1 mM p-nitrothiophenol, 2,2 mM imidazole, 7 hrs, 45°] gave 32 % of an amorphous product, which was purified by chromatography on Sephadex LH-2o in methanol. The substance gave only one spot in tlc, was nihydrin-negative and showed after hydrolysis all aminoacids in the expected ratio. Its molecular weight was correct as checked by mass spectrometry (M^* = 5o2) [2].

2) NPS-leucyl-valyl-diprolyl-alanyl-phenylalanine-(p)-nitrothiophenylester was prepared by coupling of NPS-leucine with the trifluoracetate of the corresponding pentapeptideester. After purification by preparative tlc the cyclisation was carried out in anhydrous pyridine [o,1 mM, 5o ml pyridine, 1,5 mM p-nitrothiophenol, 3 mM imidazole, 4o°, 3 hrs.] to give at least 2o % of cyclo-(leucyl-valyl-diprolyl-alanyl-phenylalanyl). An analogous cyclisation experiment at room temperature in anhydrous tetrahydrofurane [o,125 mM, 6o ml THF, 1,6 mM p-nitrothiophenol, 3 mM imidazole, 25°, 2o hrs] led to the same result. Purification of this cyclopeptide was simplified by filtering the crude, dried reaction mixture in 3oo ml of benzene through a 2 x 1o cm column of silica gel (Merck o,o5 - o,2 mm) followed by 3oo ml of ethyl acetate and 3oo ml of tetrahydrofurane that finally washed out the peptide from the silica gel. A small amount of imidazole simultaneously eluted was removed by washing the ethylacetate solution with 1 N hydrochloric acid saturated with sodium chloride.

Still better results, however, were obtained, when the cyclisation was carried out without imidazole, the reaction being started by splitting off the NPS-residue with p-nitrothiophenol [1]; here the yield of cyclopeptide was at least 28 % [o,1 mM, 45 ml pyridine, 1,5 mM p-nitrothiophenol, 4o°, 5 hrs]. The cyclic peptide again was identified by tlc, aminoacid analysis and mass spectrometry (M^* = 624), m.p.: 195°.

3) Finally a cyclic decapeptide, antamanide [3], was synthetized from the corresponding linear starting material. This, NPS-phenylalanyl-diprolyl-diphenylalanyl-valyl-diprolyl-alanyl-phenylalanine-(p)nitrothiophenylester, was prepared by condensation of

two pentapeptides or, with better yield, by reaction of NPS-chloride with the free decapeptide in pyridine and reacting its mixed ethyl carbonic anhydride with p-nitrothiophenol. After purification by tlc the cyclisation was carried out yielding 3o % of antamanide (o,o5 mM in 25 ml anhydrous pyridine, o,32 mM p-nitrothiophenol, 1,5 mM imidazole, $4o^{o}$, 3 hrs.].

The cyclopeptide again was found in the tetrahydrofuran extracts, when filtered through silica gel, after the yellow byproducts had been washed out with benzene and ethylacetate. Antamanide prepared by this method had m.p. 172^{o} and proved to be identical with the natural product and the synthetic ones prepared by other routes [4].

LITERATURE

[1] H. Faulstich, Chimia 23, 15o (1969).

[2] We wish to thank Dr.W.Otting, Heidelberg, and Dr. A.Prox, München, for mass spectrometric analysis. The fragmentation data will be published later.

[3] Th.Wieland et Al., Angew.Chem. 8o, 2o9 (1968); Internat.Ed. 7, 2o4 (1968).

[4] Th.Wieland, J.Faesel and W.Konz, Liebigs Ann.Chem. 722, 197 (1969).