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IMPROVED SOLID PHASE PEPTIDE SYNTHESIS II. THE REACTION OF AN α-AMINO ACID N-CARBOXYANHYDRIDE WITH N-TRIMETHYL SILYL AMINO ACID NON-CROSSLINKED RESIN ESTER

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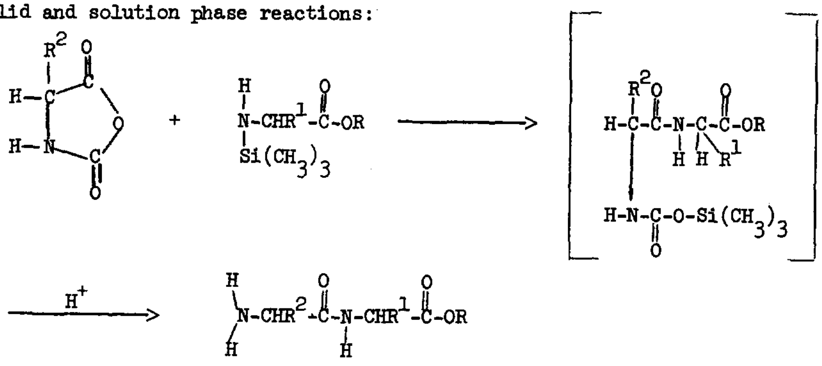
In a previous communication we have described the use of a non-crosslinked resin as a support for peptide synthesis (1). We have extended our work in this area and now present data describing the reaction of an α-amino acid N-carboxyanhydride (NCA) with an N-trimethyl silyl (TMS) amino acid ester. The earlier applications of the N-carboxyanhydride method in solution phase peptide synthesis have dealt with the stabilization of the carbamate intermediate. This was necessary in order to prevent undesired side reactions (2) of the labile NCA. Bailey (3) used Et_3N at very low temperatures in organic solvents to stabilize the carbamate. Denkewalter (4) stabilized the intermediate as the sodium salt at 0°C in aqueous solvents; a method which gave fast reactions. Iwakara (5) reported results, similar to Denkewalter (4), in a liquid-liquid interface reaction employing acetonitrile and water.

Since our resin is hydrophobic it was not possible to use the aqueous methods of Denkewalter (4) and Iwakara (5), and therefore we adapted Bailey's (3) techniques employing organic solvents. Our initial experiments with the NCA method on the non-crosslinked resin resulted in peptide synthesis. However, the efficiency of the reaction and control of over-reaction were critically dependent on temperature. We felt that this approach had promise if we could stabilize the carbamate intermediate in a way that would allow efficient peptide synthesis. Toward this end we explored the use of the trimethyl silyl group. Silylated amino acids have been used in the following coupling reactions: phosphorous oxychloride (6), imidazolid (7), mixed anhydride (8-10). In addition Oertel (11) has described the reaction of a silyl amine with a cyclid anhydride (11). In a recent publication Kreicheldorf (12) has described the solution phase synthesis of amides, in the reaction of an α-amino acid N-carboxyanhydride with various N-silylated primary and secondary amines.

We felt that the NCA would react with the N-TMS amino acid non-crosslinked resin ester and the silyl carbamate formed would be stable enough to prevent the undesired over-reaction of more than one residue adding per coupling cycle (2).

We have synthesized dipeptides in both the solid and solution phases at room temperature by reacting an α-amino acid N-carboxyanhydride with an N-trimethyl silyl amino acid ester. The solid phase synthesis involves the reaction of the NCA with N-TMS amino acid non-crosslinked resin ester (1). In solution phase the NCA is reacted with N-TMS amino acid ethyl ester.

On the basis of previous NCA studies (3, 11, 12) we propose a general scheme for both the solid and solution phase reactions:



R = non-crosslinked resin in solid phase

R = C_2H_5 in solution phase

In solid phase reactions, the amino acid non-crosslinked resin esters of valine and glycine were prepared and deblocked (1). The N-TMS derivative was prepared by reacting the amino group with an excess (20% or greater) of Et_3N and $(CH_3)_3SiCl$ in $CHCl_3$ for 30 min. at room temperature, similar to the procedure of Hillmann (13). 100 mg of N-TMS valine resin ester (.7 mm val/gm of resin) was reacted with 0.143 mm of L-leucine NCA (14) in a Merrifield type vessel for 90 min. in 5 ml of $CHCl_3$. The solution was filtered and the resin washed: 3 x 10 ml $CHCl_3$. Amino acid analysis of the resin hydrolysate showed the following values: (Val 0.703 mm and Leu 0.698 mm) per gm of resin, and a ratio of: Val 1.00, Leu 0.995.

Similarly, L-leucine NCA (0.56 mm) was reacted with 100 mg of N-TMS glycine resin ester (0.14 mm gly/gm of resin) for 90 min. Identical work up and anlysis gave value: (gly 1.39 mm and Leu 1.34 mm) per gm of resin and a ratio of: Gly 1.00, Leu 0.956. Analyses were performed on the Beckman 120C Amino Acid Analyzer.

In the solution phase identical reactions, a and b, were run but worked up differently. In each 1.57 g (0.01 m) of L-Leucine NCA and 1.75 g (0.01 m) of N-TMS glycine ethyl ester (13) were mixed in 10 ml of dry C_6H_6 at room temperature.

In reaction a, the nmr, Varian A-60, of the reaction mixture, taken within 5 min after mixing, showed a complete disappearance of the TMS absorption of N-TMS glycine ethyl ester at -33 cycles and the appearance of TMS absorption at -21 cycles. The nmr showed no further changes on standing an additional 30 min. EtOH, 30 ml, was added and the solution concentrated under hi vacuum, to give an oil, nmr of the oil in dry C_6H_6 did not have a TMS absorption, indicating decomposition of the proposed carbamate by EtOH. The ester was hydrolysed to the acid in 5 ml N NaOH. After neutralization with AcOH, the product was concentrated in vacuum and recrystallized from eg EtOH to give 1.63 g (86%) of L-leucyl-glycine. The structure was confirmed by mp 246°C, (lit. 248°C), $[\alpha]_D^{22}$= +80.3,Lit. $[\alpha]_D^{20}$= 81.5 and ir comparison with the known dipeptide (15).

In reaction b, treatment of the reaction mixture with 10 ml of Et_2O saturated with HCl caused the evolution of gas bubbles. After 30 min the material was concentrated under vacuum to an oil.A tlc sample Rf 0.53 (PrOH-H_2O, 70/30) was identical to L-leucyl-glycine (15) which was esterified with $SOCl_2$ in EtOH. The product was purified by precipitation from EtOH by Et_2O, and repeated washing of the oil with EtOAc, then dried to give 2.27g (91%) of Ethyl-leucyl-glycinate HCl. Anal. Calcd. for $C_{10}H_{21}O_3N_2Cl$: C, 47.6; H, 8.34; N, 11.01. Found: C, 48.30; H, 8.57; N, 10.43.

These experiments have shown that this new method of solid phase peptide synthesis offers the mild and facile removal of the TMS protecting group (1% AcOH in EtOH). This avoids the more rigorous acid (HCl or THF) deblocking methods, and their disadvantages (16, 17) common to other methods of solid phase peptide synthesis.

We have recently completed the solid phase synthesis of the nonapeptide oxytocin by the NCA-TMS method, and have found it to be both optically and biologically active. A detailed report of this work is in progress.

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