Tetrahedron Letters No. 28, pp. 2633-2636, 1971. Pergamon Press. Printed in Great Britain.

A NEW SOLID SUPPORT FOR POLYPEPTIDE SYNTHESIS

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(Received in USA 21 May 1971; received in UK for publication 15 June 1971)

The discovery of the solid-phase synthesis by Merrifield (1) opened a new way in the synthesis of polypeptides. However, since the chlorobenzyl-groups are uniformly distributed throughout the polystyrene-resin particles (2), all reaction rates are unfavorably controlled by diffusion. This distribution cannot be overcome by using resins with lower concentration of chlorobenzyl groups. A second difficulty arises from using different solvents during reaction and washing steps which cause different swelling of resin particles, thus affecting reaction rate, yields in coupling steps, and purity of synthesized peptides (3,4). Differential swelling of the polymer particles also causes difficulties in the automization of this process, and therefore batch procedures must be used instead of column procedures which would be more convenient and more easily controlled (5).

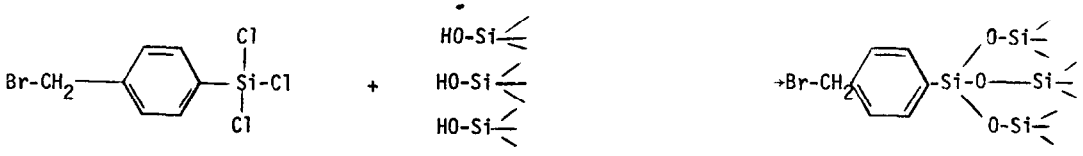
All of these difficulties can be overcome by using inorganic support material coated with a thin layer of molecules which are chemically bonded to an inorganic surface and have reactive groups located only on the surface. Esterification with 1,4-dihydroxydimethyl benzene was chosen by Bayer et al. (5,6) for formation of the chemically bonded monomolecular layer on which peptides can be built by stepwise synthesis. The advantage of this approach were proven by syntheses of the dodecapetide $(leu\text{-}Ala)_6$ and the tetrapeptide (leu-leu-glu-gly). All reaction times were reduced in comparison with the polystyrene-based resins. No failure sequences could be detected in the test peptide $(leu\text{-}Ala)_6$.

The main disadvantage with this support is that the Si-O-C bond is highly polarized and thus very sensitive to attack by all reagents containing free hydroxy groups, but especially towards water. This difficulty can be overcome by bonding organic molecules to siliceous surfaces through Si-O-Si-C bonds which are more stable against an attack by electrophilic or nucleophilic agents than are the Si-O-C bonds.

Si-O-Si-C bonds can be formed by reaction of surface silanol groups with compounds of formula R_xSiY_{4-x} (R = alkyl, aryl, Y = halogen, X = 1-3) (7). These reactions have been used for formation of chemically bonded layers of organic molecules on surface of siliceous materials in the field of gas and liquid chromatography (8,9). In order to obtain such material for the synthesis of peptides, we prepared p-bromomethyl-phenyl-silica. P-tolyl-magnesium bromide was reacted with silocon tetrachloride according to the method described by Chvalovsky et al. (10). The p-tolyl trichlorosilane obtained was brominated in CCl_4 with B-bromosuccinimide (NBS) catalyzed by benzoylperoxide according to:

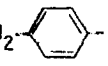
After removal of succinimide and the solvent, the oil was twice distilled in vacuo (B.P. 125°/2mm Hg). The purity and structure of the p-bromomethyl-phenyl-trichlorosilane was determined by NMR-spectroscopy.

The above compound was reacted with the silanol groups on the glass surface (Bio-Glass 2500, Bio. Rad Lab) by shaking the beads overnight with 5% solution of p-bromomethyl-phenyl-trichlorosilane in benzene. After filtration the coated glass beads were washed with benzene, a mixture of benzene and ethanol (1:1) and finally with benzene.



The remaining Si-Cl bonds were hydrolyzed by mixture Water: Ethanol: Benzene (5:45:50). The silicone layer was then polymerized by heating to 100°C for 24 hours in vacuo. The silanized glass beads were washed with ethanol and benzene in order to remove absorbed impurities. The capacity of silanized glass beads was determined by modified Volhard analysis for bromine (0.08 mmoles Br/g glass). At this point no chlorine could be detected. In order to demonstrate suitability of this support for solid phase synthesis of peptides, we have synthesized a model peptide H-Pro-Gly-Phe-Ala-OH.

The first amino acid, alanine, was connected with the carboxyl group to benzylic group of silicone layer by refluxing the silanzied glass beads in dioxane with a solution of triethylammonium salt of t-Boc amino acid for 24 hours. The amino acid thus bonded to a support were not affected by either trifluoroacetic acid and mixture of triethylamine/chloroform for a period of longer than 20 hours. No free amino acid could be detected in either reagent by thin layer chromatography. Further, the amino acid was not affected by the action of all common solvents used in solid phase synthesis. N,N'-dicyclohexylcarbodiimide (DCC) and CH_2Cl_2 was used for coupling of t-Boc amino acids to alanine amino attached to glass support. Reaction time for each coupling step was 2 hours at room temperature. For deprotection and washing we have used standard procedure described by Merrifield (1). The finished tetrapeptide was cleaved from the support by HBr/trifluoroacetic acid for 2 hours at room temperature. The identity of finished peptide was confirmed by amino acid analysis. Acid hydrolysis with 6NHCl for 24 hours at 110°C of the isolated tetrapeptide showed the following molar ratio: Pro 0.95, Gly 1.00, Ala 1.00, Phe 1.05 (Gly being taken as 1.00). The tetrapeptide showed a single spot by thin layer chromatography.

However, cleavage of the peptide from the support did not proceed to completion. Total hydrolysis of peptide remaining on the support, by amino acid analysis showed that cleavage step proceeded only to about 50% conversion. This is caused by negative effect of electron withdrawing SiO_3 group in para position toward a benzylic group. In order to overcome those difficulties we have synthesized several silanes of X-CH_2-  -$(CH_2)_n$-$SiCl_3$ type (n = 2,3,4), X = halogen. The applications of these compounds for peptide synthesis

will be studied.

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