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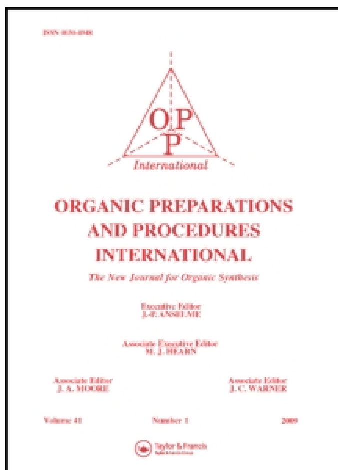

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MACROPOROUS POLYSTYRENE BEADS AS A NEW RIGID SUPPORT FOR SOLID PHASE PEPTIDE SYNTHESIS

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MACROPOROUS POLYSTYRENE BEADS AS A NEW RIGID SUPPORT FOR SOLID PHASE PEPTIDE SYNTHESIS

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Three different types of polymers have been reported as useful supports in solid phase peptide synthesis: microporous polystyrene resins[1], phenolformaldehyde[2], and soluble polystyrenes.[3]

The generally accepted concept in solid state peptide synthesis using microporous resins is that most of the peptide chains are restricted to the internal matrix of the polymer, with few of the reactive sites on the outside surface.[1,2] This concept is supported by studies on the hydrolytic cleavage of amino acids and peptides attached to the resin in the conventional Merrifield manner. Hydrolysis using aqueous 6N HCl cleaves less than 10% of the attached amino acid or peptide. In contrast, hydrolysis in the presence of a solvent such as dioxane which causes the resin to swell releases nearly 100% of the amino acids or peptides attached to the resin. This indicates that most of the attachment sites are in the internal matrix of the resin and that the resin must be in the swollen state for the sites to be readily accessible.

The recent introduction of macroporous cross-linked polystyrenes for use in size separations of non-ionic

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species prompted us to investigate their use as a solid phase support in peptide synthesis. Because of their very rigid internal matrix, the cavities may have cross dimensions as high as 200 A°. Thus, macroporous resins should have a higher percentage of the reacting sites accessible in the non-swollen state than the microporous resin which is usually used in Merrifield synthesis. This could possibly allow reactions to be carried out in solvents which do not produce a swelling of the resin and polar but non-swelling solvents such as water, alcohols and carboxylic acids should be utilizable for washing ionic side products and reactants from the resin. The swelling and shrinking of microporous resin due to temperature and solvent changes may cause residues attached to certain parts of the polymer chains to become permanently or, even worse, temporarily unavailable for reaction. This can contribute to heterogeneity of the product. In this respect, macroporous resin, being rigid, may offer an advantage.

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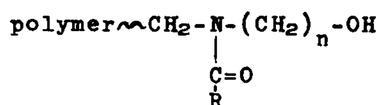
Chloromethylation of macroporous polystyrene SM-1 resin (Calbiochem, Los Angeles) was performed by stirring 20 g. of the dried beads (dried by washing with EtOH, dioxane and CH_2Cl_2 and evacuating in an oven) in 150 ml nitrobenzene at 10°C and slowly introducing a mixture of 3.6 ml of chloromethyl methyl ether and 4 ml $SnCl_4$ in 15 ml nitrobenzene. The mixture darkened, and stirring was continued at room temperature for 1.5 hrs. The resin was filtered and washed 3 times each with 100 ml of 3:1 dioxane:3N HCl, 3:1 dioxane: H_2O, dioxane, and CH_2Cl_2, and then was dried _in vacuo_.

Elemental analysis gave Cl 5.12%. Repetition of this procedure gave identical results. Using the coupling technique of Merrifield, t-BOC-L-Phe was substituted to the extent of 0.22 mMoles/g. resin. When this t-BOC-L-Phe substituted resin was hydrolyzed with aqueous 6N HCl, the yield of amino acid was 60% as compared to acid hydrolysis of the polymer ester in the presence of dioxane which was 100%.

After deprotection and neutralization, the phenylalanine esterified to the macroporous polymer was used for the synthesis of alanyl phenylalanine using a standard Merrifield solid phase technique: a 4-fold excess of t-BOC alanine and dicyclohexylcarbodiimide (DCC) activation. The peptide attached to the polymer was acid hydrolyzed in the presence of dioxane and showed one to one ratios of Ala and Phe. The peptide was removed from the resin by saponification, deprotected with HBr in acetic acid and identified as Ala-Phe by comparison with authentic Ala-Phe on an amino acid analyzer adapted for peptide analysis. No phenylalanine was detected, which indicates quantitative coupling.

We have also synthesized modified macroporous polystyrene resins of the type



similar to the derivatives described for microporous polymers[4] which permit the use of N-α-carbobenzoxy (N-α-Cbz) amino acid derivatives in the solid phase peptide synthesis. Using N-α-Cbz amino acids in four-fold excess, and DCC activation,

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we have now synthesized beef insulin B-chain C-terminal tetrapeptide. O-acetyl and trifluoroacetyl groups were used for side chain protection of Thr and Lys respectively.[5] Analysis of an acid hydrolysate of the peptide indicated amino acid ratios of Ala 1.1, Lys 1.0, Pro 1.0 and Thr (uncorrected) 0.8.

Our experience shows that the macroporous resin is suitable for synthesis of dipeptides and possibly suitable for oligopeptides. The properties of large, non-swelling internal cavities and compatibility with polar solvents such as water may make this resin a suitable choice for further exploration in solid phase peptide chemistry.

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