Tetrahedron Letters No.11, pp. 1297-1300, 1968. Pergamon Press. Printed in Great Britain.

POLYSTYRENE MODIFICATIONS FOR SOLID PHASE PEPTIDE SYNTHESIS WHICH ARE COMPATIBLE WITH N-α-CARBOBENZOXY PROTECTION AND MIXED ANHYDRIDE ACTIVATION

Manohar A. Tilak and C. Stephen Hollinden

Lilly Research Laboratories, Eli Lilly and Company
Indianapolis, Indiana

(Received in USA 20 November 1967)

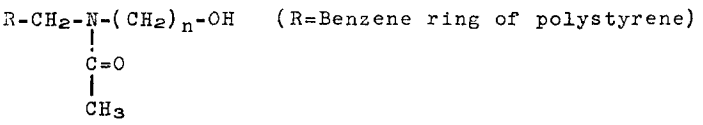
Since the introduction of solid phase synthesis by Dr. R. B. Merrifield (1), many peptides have been synthesized using this new technique (2,4,5,6,7,8,9). In the initial work on solid phase synthesis (2,4), the use of N-α-carbobenzoxy (N-α-CBZ) protection (3) was only partly successful because of insufficient stabilization of the benzyl ester (2). All subsequent solid phase syntheses reported have utilized N-α-t-butyloxycarbonyl (N-α-t-BOC) (10), N-α-O-nitrophenyl sulphenyl (11), or N-α-p-methoxy-carbobenzoxy (13) protecting groups.

Coupling has been accomplished with active esters (e.g. p-nitrophenyl (8), N-hydroxy succinimid (14), etc) or N,N'-dicyclohexylcarbodiimide (DCC) (15). These methods of activation usually require long reaction times for quantitative coupling (13,18) unless a large excess of reactants is used.

Mixed anhydride activation, which allows short coupling periods even at low temperatures (-15°C) and easy work up procedures (due to easily separable by-products), and which offers steric advantages (compared to other activations) has not been reported in solid phase peptide synthesis.

We now report the synthesis of two new types of polystyrene derivatives useful in conjunction with the readily available and relatively inexpensive N-α-CBZ-amino acid derivatives and with mixed anhydride activation. The

first is a polymer derivative of the type:



For example, amino ethanol and 1,6 amino hexanol derivatives were obtained by reacting at least a 20 fold excess (based on Cl) of the corresponding amino alcohol with chloromethylated polystyrene resin (Biobeads S-X2 (16) containing 3-5% Cl) in hot dioxane (10 ml/g) for several hours. The resin was washed thoroughly to remove excess amino alcohol. Complete substitution of halogen by amino alcohol was confirmed by elemental analysis for N and Cl. The resin was then suspended in dioxane and reacted overnight at room temperature with 80 fold excess of acetic anhydride (based on original Cl). The corresponding N-acyl resin derivative was confirmed by a tertiary amide peak at 1640 cm^{-1} in the infrared spectrum (IR) of the KBr pellet. There was also a small ester peak at 1750 cm^{-1}. The ester peak disappeared after saponification for 2 hours at room temperature with 3 moles excess of aqueous NaOH (diluted in sufficient ethanol:dioxane (1:1) to form a single phase) while the tertiary amide peak remained. The resin alcohol group was then esterified with N-α-CBZ phenylalanine using carbonyl diimidazole (12,8). (Attempts to esterify the resin hydroxyls using DCC or mixed anhydride activation were not successful.) Incorporation of phenylalanine (0.15 to 0.35 mmoles/gm) was confirmed by amino acid and IR analyses.

The removal of the N-α-CBZ group with 30% HBr in acetic acid was complete within 30 minutes. The deprotecting reagent was then filtered off and the resin was washed thoroughly with acetic acid and then with CH_2Cl_2. The IR of the resin showed disappearance of the urethane absorption at 1720 cm^{-1} and persistence of the ester peak at 1750 cm^{-1}. Thin layer chromatography of the concentrated filtrates failed to show any amino acid, under conditions in which 0.1% ester cleavage would have been detected.

The amino acid ester hydrobromide resin obtained above was neutralized with excess triethylamine, and the product was carefully washed with CH_2Cl_2. Peptide coupling was carried out at -15°C (or as low as -35°) for 2-3 hours using one mole excess of the isobutyl chloroformate mixed anhydride of N-α-CBZ-alanine.

After the peptide coupling, mild saponification (less than 2 fold excess of 0.05 N aqueous NaOH in dioxane:ethanol 1:1) gave a 97% yield of Ala-Phe. The identity of the peptide was confirmed by comparison with authentic Ala-Phe using thin layer chromatography and retention times on ion exchange columns of an amino acid analyzer adapted for peptide analysis. Free alanine was not detected, indicating that none of the second amino acid was esterified to the unreacted resin hydroxyls. Acid hydrolysis of the isolated dipeptide showed both amino acids in a 1:1 ratio.

The second polymer derivative prepared for use with N-α-CBZ protection is of the type: $R\text{-}\overset{\overset{O}{\|}}{C}\text{-}(CH_2)_3\text{-}OH$ (R=Benzene ring of polystyrene)

One of these was synthesized by the Friedel-Crafts reaction of ω-chlorobutyryl chloride (12 mmoles) and $AlCl_3$ (24 mmoles) with cross-linked polystyrene resin (6 gm) (Biobeads S-X2 (16)), in 80 ml of nitro benzene for 1 1/2 hour at 5°C. After washing with AcOH, 6 N HCl-dioxane (1:1), H_2O-dioxane (1:1), dioxane and CH_2Cl_2, the product was boiled for several hours with a large excess of triethylamine (1 gm 10 mmoles) and acetic acid (11 mmoles) in ethanol (30 ml) for 30 hours. After dioxane washing and subsequent saponification (see ex. 1) of the ester formed in this reaction, the alcohol on the resin was esterified with N-α-CBZ phenylalanine using carbonyl diimidazole activation. This type of ester linkage was stable to the acid reagents used for decarbobenzoxylation. High yields of peptide coupling (90% Ala-Phe) were obtained using this resin and mixed anhydrides.

Work on the synthesis of longer peptides using the above resins, N-α-CBZ protection, and mixed anhydrides is in progress.

Acknowledgements:

The authors are indebted to Dr. W. W. Bromer for encouragement. Thanks are due to Mrs. Mary Lynn Hendricks for excellent technical assistance.

REFERENCES

1. R. B. Merrifield, Federation Proc. 21, 412 (1962).
2. R. B. Merrifield, J. Am. Chem. Soc. 85, 2149 (1963).
3. M. Bergmann and L. Zervas, Chem. Ber. 65, 1192 (1932).
4. R. B. Merrifield, Biochemistry 3, 1385 (1964).
5. R. Marshall and R. B. Merrifield, Biochemistry 4, 2394 (1965).
6. D. W. Wooley, J. Am. Chem. Soc. 88, 2309 (1966).
7. V. A. Najjar and R. B. Merrifield, Biochemistry 5, 3765 (1966).
8. M. Bodansky and J. T. Sheehan, Chem. and Ind. 38, 1597 (1966).
9. A. Marglin and R. B. Merrifield, J. Am. Chem. Soc. 88, 5051 (1966).
10. G. W. Anderson and A. C. McGregor, J. Am. Chem. Soc. 79, 6180 (1957).
11. L. Zervas and C. Hamalidis, J. Am. Chem. Soc. 87, 99 (1965).
12. H. A. Staab, Angew. Chem. 71, 194 (1959).
13. F. Weygand and W. Ragnarsson, Z. Naturforschg 21b, 1141 (1966).
14. G. W. Anderson, J. E. Zimmerman and F. M. Callahan, J. Am. Chem. Soc. 86, 1839 (1964).
15. J. C. Sheehan and G. P. Hess, J. Am. Chem. Soc. 77, 1067 (1955).
16. Purchased from Bio-Rad Laboratories, Richmond, California.
17. J. R. Vaughan and R. L. Osato, J. Am. Chem. Soc. 74, 676 (1952).
18. Private Communication F. Weygand (Munich).