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A FACILE METHOD FOR THE SYNTHESIS OF BENZYL ESTERS USING BENZYL BROMIDE OR IODIDE AND ITS APPLICATION TO SOLID PHASE AND CONVENTIONAL PEPTIDE SYNTHESIS; ATTEMPTED STERICAL SELECTION IN SOLID PHASE SYNTHESIS.

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1. In the Merrifield solid phase peptide synthesis (1), the benzyl ester attachment of the first amino acid residue to the chloromethylated polystyrene has customarily been effected by heating the polymer, the protected amino acid, and a tertiary amine in an inert solvent at 80° for 48 hours.

Bodanszky and Sheehan (2) have reported a milder esterification procedure in which the protected amino acid must be activated separately with carbonyl diimidazole and then reacted with the hydroxymethyl form of the polymer. This procedure is not applicable to either hydroxy amino acids due to possible self condensation or glutamine and asparagine derivatives which may dehydrate to nitriles. The hydroxymethyl polymer was obtained by esterification of the chloromethylated form with potassium acetate followed by saponification. Another esterification procedure using methyl-sulfonium polystyrene, which has been reported recently, may have some advantages (3).

1.1 We now report a procedure for esterification of the polymer under mild conditions using the benzyl bromide or benzyl iodide form of the polystyrene resin. In this mild procedure, there is no danger of racemization since activation of the carboxyl group of the protected amino acid is not required (the alcohol component itself being activated).

The benzyl bromide and iodide forms of the polystyrene resin (obtained from the ester form by treatment with HBr or HI in AcOH) were found to react in a few hours at room temperature with protected amino acids in the presence of a base. N-α-t-butyloxycarbonyl (t-BOC) (4), o-nitrophenylsulphenyl (o-NPS) (5), and 1,3-diketone enamine protected amino acids have been used with success in our experiments as shown in Table I.

1.2 Preparation of Bromo or Iodo Methyl Polystyrene Resin

Five grams of chloromethylated polystyrene resin (Biobeads S-X2, 3-5% Cl) were heated with 25 mmoles of AcOH (t-BOC amino acids or pivalic acid were also used to get a limited amount of Br or I into sterically favored positions) and 25 mmoles triethylamine in 50 ml dioxane at 80° for 18 hours. After washing repeatedly (AcOH, alcohol, and dioxane, three washes with 50 ml of each solvent) and drying, the esterification of the resin was confirmed by a peak at 1750 cm^{-1} in the infra-red spectrum (IR). The esterified resin was stirred for 1/2 to 1 hour with an excess of HBr or HI in AcOH and then was washed with AcOH and CH_2Cl_2. Formation of the bromo- or iodo-benzyl resin was confirmed by the disappearance of the ester in the IR and by elemental analysis which indicated 4% Br or 9% I, respectively.

1.3 Esterifica ion of the Resin

The benzyl bromide or iodide type polystyrene beads were stirred overnight at room temperature with the N-protected amino acid and the base in DMF or CH_2Cl_2. After filtration and repeated washing of the polymer with various solvents (CH_2Cl_2, DMF, alcohol), the presence of the ester was confirmed by the IR and amino acid analysis.

The level of bromine or iodine substitution on the polymer can be controlled by limiting the original halogen-methylation, the degree of esterification, or the amount of HBr or HI used to derive the benzyl bromide or iodide form of the polymer. Pivalic acid and t-BOC-amino acids because of their own sterical requirements would be expected to react only in the sterically favored positions. This was confirmed by using benzyl Br or I resins obtained from AcOH esters. In this case only one-third of the Br or I could be substituted with amino acids, whereas the Br or I on the resins obtained from pivalic acid or t-BOC-amino acid esters was completely substituted. (see Table I, lines 1-5) This indicates the advantage of steric selection in the original esterification.

1.4 Peptide Synthesis on Polymer Support

Iodomethylated polymer prepared by treatment of pivalic acid resin ester with HI was reacted with carbobenzoxy-L-phenylalanine. Deblocking and coupling

with carbobenzoxy-L-alanine by the mixed carbonic anhydride procedure (6) afforded the dipeptide in 94% yield. The identity of this dipeptide was confirmed by comparison with a standard preparation of the same dipeptide using an amino acid analyzer adapted for peptide analysis.

2. Benzyl Ester Preparation for Use in Conventional Peptide Synthesis

Using a similar approach we have also devised a convenient method for preparation of benzyl esters of protected amino acids which is compatible with acid and base labile protecting groups. Benzyl esters of N-α-carbobenzoxy (N-α-CBZ) amino acids are usually prepared by refluxing them with benzyl alcohol in the presence of p-toluene sulfonic acid (catalyst) using azeotropic systems for the removal of water (7), a procedure which in all probability is not compatible with protective groups such as t-BOC, N-α-benzhydryloxycarbonyl (8), enamine (9), or o-NPS.

In our procedure the protected amino acid and an equivalent amount of dicyclohexylamine (or other bases) dissolved in DMF or a CH_2Cl_2/DMF mixture, are stirred overnight at room temperature with an equivalent amount of benzyl bromide or iodide. After evaporation to dryness in vacuo, trituration, and washing of the residue with ethyl acetate, the filtrate is shaken with cold 1N HCl (where permissible), water, sodium bicarbonate, and NaCl solutions. After drying and evaporation to a small volume, petroleum ether is added to crystallize the product. Since the benzyl ester formation takes place under such mild conditions, this method can be used even with amino acids protected with acid and base labile groups.

The following esters had excellent C, H, N, and O analyses.

CBZ-Gly-Bzl ester: $C_{17}H_{17}NO_4$ (299.31), 95% Anal. pure yield, mp 69-71°C (Lit. 72) (10), Infra-red (IR) absorption at 1750 cm^{-1} (ester).

CBZ-L-Ser-Bzl ester: $C_{18}H_{19}NO_5$ (329.34), 70% Anal. pure yield, mp 81.5-83.5°C (Lit. 84-85) (10), IR peak 1760 cm^{-1}, $[\alpha]_D^{25}$° =+5.64° Conc. 4%, $CHCl_3$ (Lit. +5.7)(10).

CBZ-L-threonine-Bzl ester: $C_{19}H_{21}NO_5$ (343.39), 71% Anal. pure yield mp 76-78°C, $[\alpha]_D^{25}$° C=-13.7° Conc. 4%, $CHCl_3$. (New Compound)

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TABLE I

Benzyl Ester Formation Using Benzyl Br or I Form of Polystyrene Resin

Acid Used for Original Esterification of Resin	Level of Original Ester (mmoles/g of Resin)	Type of Polymer Modification: Benzyl Br or I	Protected Amino Acid Used: Ratio of Halogen to Protected Amino Acid	Base Used	Amino Acid Incorporated on to Resin (mmoles/g of Resin)
Pivalic Acid	0.21	I	1:6 t-BOC-L-Phe	DCHA*	0.209 Phe
t-BOC-L-Phe	0.23	Br	1:4 t-BOC-Gly	DCHA	0.22 Gly
t-BOC-Gly	0.06	Br	1:8 t-BOC-L-Asn	DCHA	0.063 Asp
t-BOC-Gly	0.06	Br	1:8 t-BOC-Gly	TEA*	0.06 Gly
t-BOC-Gly	0.06	Br	1:8 t-BOC-Gly	DEA*	0.067 Gly
Acetic Acid	0.9	Br	1:2 t-BOC-Val, Na^+	---	0.133 Val
Acetic Acid	0.9	Br	1:3 t-BOC-L-Phe	DCHA	0.245 Phe
Acetic Acid	0.9	Br	1:3 BzAc Enamine-L-Phe, DCHA	---	0.257 Phe
Acetic Acid	0.9	Br	1:3 o-NPS-L-Ala	DCHA	0.218 Ala
Acetic Acid	0.9	Br	1:1.5 t-BOC-Val	DCHA	0.212 Val
Acetic Acid	0.8	I	1:2.5 t-BOC-Phe	DCHA	0.316 Phe

*DCHA=Dicyclohexylamine, TEA=Triethylamine, DEA=Diethylamine

REFERENCES

1. R. B. Merrifield, J. Am. Chem. Soc., 85, 2149 (1963).

2. M. Bodansky and J. T. Sheehan, Chem. and Ind., 38, 1597 (1966).

3. L. C. Dorman and J. Love, 155th National Meeting, Amer. Chem. Soc., Abstr. p. 146, 1968.

4. G. W. Anderson and A. C. McGregor, J. Am. Chem. Soc., 79, 6180 (1957).

5. L. Zervas and C. Hamalidis, J. Am. Chem. Soc., 87, 99 (1965).

6. M. A. Tilak and C. S. Hollinden, Tetrahedron Letters, 1297 (1968).

7. D. Ben Ishai, and A. Berger, J. Org. Chem., 17, 1564 (1952).

8. R. G. Hiskey and J. B. Adams, Jr., J. Am. Chem. Soc., 87, 3969 (1965).

9. E. Dane, F. Dress, P. Konrad and T. Dockner, Angew. Chem., 74, 783 (1962).

10. J. P. Greenstein and M. Winitz, Chemistry of the Amino Acids, Vol. 2, p. 953. John Wiley and Sons, New York (1961).