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Zinc-Salt Assisted Anchoring of Carboxylic Acids to Merrifield Resin

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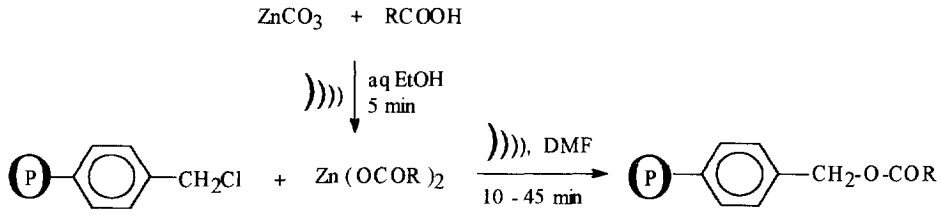
Abstract: Zinc salts of carboxylic acids were readily obtained by sonication of the acid in aqueous alcohol with zinc carbonate. Reaction of the zinc salts with chloromethylpolystyrene (Merrifield resin), again accelerated by ultrasound, yielded anchored benzyl esters useful for building peptides. The reaction was also used for rapid determination of benzylic chlorine on the resin to monitor the extent of substitution.

Anchoring of carboxylic acids onto polymers is an important step in solid-phase peptide synthesis[2]. Chloromethylated polystyrene (Merrifield polymer) and hydroxymethylpolystyrene (obtainable from acetoxymethylpolystyrene) have been traditionally used as starting materials. Among the various methods available for the purpose, use of the Merrifield polymer and cesium salts of N-butyloxycarbonyl(Boc)amino acids has been most popular, though the cesium salts are relatively expensive and the reaction is slow, usually requiring 16-24 h at 50°C in dimethylformamide[3]. The benzyl ester linkage is also the preferred mode of attachment of the first amino acid in the more recent Sheppard scheme using N-9-fluorenylmethyloxycarbonyl(Fmoc)amino acids and appropriately modified polyamide resins[4,5].

Early in 1980s, we had demonstrated that S_N1-active halides (that is, tertiary alkyl, allyl and benzyl halides) readily undergo solvolysis in presence of zinc salts to yield substitution products in high yields[6,7]. The reaction has been subsequently extended to substitution at tertiary and benzylic centres under nonsolvolytic conditions also and has been used for the preparation of a variety of t-butyl esters[8], thioethers and thiolesters[9] as well as azides[10]. However, preparation of zinc salts of carboxylic acids, required for the synthesis of esters, is cumbersome because of the insolubility of both zinc carbonate and most organic solid carboxylic acids in water. The zinc halides formed in the reaction are also a source of complication, leading to elimination products in the case of tertiary halides and Friedel-Crafts reactions in the case of benzylic halides; this could be effectively taken care of using an equivalent amount of an organic base like pyridine, but then the reactions are slow (100 h) and incomplete (70%)[8]. This reaction has now been found to be very convenient for anchoring N-protected amino acids on chloromethylated polystyrene resins for solid-phase peptide synthesis. The benzylic halides, being anchored to the polymeric backbone, do not cause complications due to Friedel-Crafts reactions unless the solvent is also aromatic. Both preparation of the zinc salts and the substitution reactions were considerably accelerated using ultrasound (5-45 min).



The suitability of the reaction for anchoring carboxylates to chloromethylated cross-linked polystyrene beads was first examined using zinc acetate (4.8 equiv.). Dimethylformamide, in which the resin beads swell appreciably, was used as the solvent. The reaction was complete in about 3h, as determined by the residual chlorine on the resin. Having recently explored the use of ultrasound in peptide synthesis[11,12], we also examined the acceleration of this reaction by sonication and found that the reaction was complete in 10 min using the same amount of zinc acetate. The results were comparable when sonication was carried out using a bath or probe (see **EXPERIMENTAL**). The reaction also proceeded to completion when acetic acid was used as solvent, but no reaction occurred when chloroform or hexane was used. Incidentally, we found this method very convenient for determination of the displaced chlorine from the resin using modified Volhard method[13]. The usual method of displacing chlorine from the resin requires refluxing it in pyridine for about 2 h to ensure completion of the reaction [14].

For anchoring amino acids onto the Merrifield resin using this method, preparation of zinc carboxylates of protected amino acids was a prerequisite. The cesium salts are usually prepared by stirring the appropriately protected amino acid derivative with water-soluble cesium bicarbonate or carbonate in aqueous ethanol for a few minutes but no reaction occurred when zinc carbonate and the caboxylic acid were stirred together for 6 h under similar conditions. However, when the protected amino acid, dissolved or suspended in aqueous ethanol, was sonicated with equivalent quantity of zinc carbonate for 5 min, the zinc carboxylate formed completely as shown by the dissolution of the zinc carbonate and the neutral pH of the solution. The translucent solution was evaporated and dried by distilling with benzene.

For anchoring onto the resin, the salt was dissolved in dimethylformamide and sonicated with calculated quantity of chloromethylpolystyrene (Merrifield) resin. The time course of the reaction was studied using N-Boc-tryptophan (1.2 equiv.) and completion of the reaction required 45 min (Table 1).

Table 1. Time course of the reaction of N-Boc-tryptophan zinc salt (0.4 mmol) with Merrifield resin (0.7 mmol Cl) in dimethylformamide under sonication.

Time (min)	Amount of chlorine on resin (mmol/g)	Extent of reaction (%)
15	0.34	51
30	0.22	69
45	0.01	99

The amount of N-Boc-amino acids on the resin was determined both by estimation of the residual chlorine on the resin and by deblocking the amino group and its quantitation by the picric acid method[15]. Table 2 shows the results of the reaction with 20 other amino acid derivatives. Hydroxylic and phenolic groups in the carboxylic acid do not apparently need to be protected as N-Boc-threonine and N-Boc-tyrosine could be used in the reaction

without further protection. Zinc salts of Fmoc-amino acids could also be similarly prepared and used in the reaction without loss of the Fmoc group. The integrity of the amino acid residue on the resin was verified by cleaving the benzyl ester linkage by a novel method involving simultaneous base-catalysed transesterification and hydrolysis, followed by comparison of the resultant N-Boc-amino acid with authentic samples (TLC, HPLC, m.p., $[\alpha]_D$)[16]. Thus, the above findings would help acceleration of the solid-phase peptide synthesis in general. Also, the reaction with zinc acetate is useful not only for determination of resin-bound chlorine but also for preparation of acetoxymethylpolystyrene and hydroxymethylpolystyrene. The conventional method of preparation of these resins requires heating chloromethylated polystyrene with 1 M potassium acetate in 2-methoxyethanol at 130°C for 24 and 70 h respectively[2]. The above described method is also likely to be of immense utility as a general method of anchoring a variety of active groups[9,10] for use as solid-phase reagents.

Table 2. Reaction of amino acids derivatives with zinc carbonate and Merrifield resin *.

Amino acid derivative	Extent of reaction (%)	Amino acid derivative	Extent of reaction (%)
N-Boc-PheOH	97.4	N-Boc-AspOH (Bzl)	100.0
N-Boc-GlyOH	92.8	N-Boc-GluOH (Bzl)	97.4
N-Boc-ThrOH	96.4	N-Boc-ArgOH (nitro)	97.1
N-Boc-MetOH	97.0	N-Boc-SerOH (Bzl)	97.1
N-Boc-AlaOH	100.0	N-Boc-AsnOH	95.7
N-Boc-LeuOH	100.0	N-Boc-ProOH	100.0
N-Boc-IleOH	100.0	N-Boc-CysOH (Bzl)	94.3
N-Boc-TyrOH	100.0	N-Boc-LysOH (Z)	93.4
N-Boc-ValOH	97.0	N-Boc-GlnOH	100.0
N-Boc-HisOH	100.0	N-Fmoc-GlyOH	94.0

*For reaction conditions and methods of analyses, see **EXPERIMENTAL**.

EXPERIMENTAL

All reagents were of commercial quality. L-Amino acids as well as appropriately protected amino acids and solvents were obtained from Sigma Chemical Company, U.S.A. Dimethylformamide was redistilled before use. Merrifield resin (2% cross-linked polystyrene carrying 0.7 mmol/g active chlorine) was obtained from Fluka, Switzerland. Sonications were carried out using Cole-Parmer (U.S.A.) ultrasonic bath (Model 8890; tank capacity 1.91 L; sonicating frequency 47 kHz; power 80W), maintained at room temperature (30°C) or a Vibracel sonic probe (12 mm horn), supplied by Sonics & Materials Inc., U.S.A. (Model VC 300; 30 W; 20 KHz).

Reaction of zinc acetate with Merrifield resin. Merrifield resin (50 mg; 0.035 mmol Cl) was suspended in dimethylformamide (1 mL) and to this was added anhydrous zinc acetate (0.015 g; 0.082 mmol). The mixture was sonicated in a sonic bath for 10 min. The resin was filtered and washed with water, methanol and dried to yield acetoxymethylpolystyrene (50 mg), devoid of chlorine. When the reaction was interrupted after 5 min, the extent of reaction was 83%, as determined by the residual chlorine. The above reaction was repeated using a sonic probe (output control 5-amp.; 50% duty cycle; sonication time 10 min). The product, obtained as above, was devoid of chlorine.

Determination of polymer-bound benzylic chlorine. The filtrate and washings from the above experiment were pooled and made up to 25 mL in a volumetric flask and the chloride content was estimated using the modified Volhard method[13] to yield 0.035 mequiv. of chloride (100%).

Anchoring of protected amino acids on chloromethylated polystyrene. N-Boc-TrpOH (0.23 g; 0.75 mmol) in ethanol (2 mL) and water (1 mL) was treated with zinc carbonate (45 mg; 0.36 mmol) and the mixture sonicated for 10 min in the bath, when the pH of the solution became neutral. The translucent solution was evaporated under vacuum on a water bath and the residue distilled twice with benzene (5 mL) to dryness. To the dry salt (0.27 g; 0.4 mmol) in dimethylformamide (5 mL) was added Merrifield resin (1 g; 0.7 mmol Cl) and the mixture sonicated in a bath at room temperature. After every 15 min , 10 mg of the resin-bound product was treated with zinc acetate as described above for determination of residual chlorine (see Table 1). After 45 min , the resin was filtered and washed with water and methanol to yield 1.2 g of the product. 10 mg of the resin-bound product was treated with 50% trifluoroacetic acid in dichloromethane and the liberated amine determined by picric acid method as described previously[15]. The results with the 20 other protected amino acids are summarised in Table 2.

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