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Synthesis of amides and peptides using polymer-bound mixed carboxylic dithiocarbamic anhydrides

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Abstract. The use of polymer-bound mixed carboxylic dithiocarbamic anhydrides in the synthesis of amides and peptides is illustrated. The introduction of acetyl, benzoyl and *p*-nitrobenzoyl and N-protected amino acyl groups into the polymer system, and acylation and peptide synthesis reactions using these polymeric acyl transfer reagents are described. Polystyrene crosslinked with hydrophilic tetraethyleneglycol diacrylate was chosen as the support for the preparation of the polymeric reagent. The reagent was prepared by a series of polymer-analogous reactions. Polystyrene resin was functionalized to aminomethyl polystyrene. The aminomethyl resin on treatment with carbondisulphide and sodium hydroxide resulted in polystyrene-supported sodium dithiocarbamate which on reaction with an acid chloride afforded the polymer-bound mixed carboxylic dithiocarbamic anhydride. The reagent on treatment with an amine or amino acid transfers its acyl group, forming the corresponding amide or peptide. In the case of acid-sensitive amino acids and amino acids containing acid-cleavable protecting groups, mixed amino acyl dithiocarbamic anhydride resins were prepared by treating sodium dithiocarbamate resin with the anhydride of the amino acid.

Keywords. Polymer-bound anhydrides; polymeric acyl transfer reagents; polymer-analogous reaction; mixed carboxylic–dithiocarbamic anhydride.

1. Introduction

The use of polymeric reagents, that contain a reactive function attached to a macromolecular backbone, received wide attention in recent years. The polymeric reagents have the chemical properties of the bound reactive residue and the physical properties of the polymer matrix. A number of polymeric reagents have been developed since the introduction of the solid-phase peptide synthesis (Merrifield 1963, 1986). Among these, polymeric acylating reagents have found considerable application in peptide synthesis and other organic synthetic reactions. A number of polymeric acylating agents have been reported for the acylation of alcohols and amines and for the synthesis of peptides (Fridkin *et al* 1972; Patchornik and Cohen 1981; Mokotolf and Patchornik 1983; Cohen *et al* 1984). Fridkin *et al* (1972) introduced the polymeric

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reagent method of peptide synthesis which makes use of an insoluble polymer-supported amino acid active ester as the carboxyl component for the coupling to the soluble amino component (Fridkin *et al* 1965; Fridkin 1979). In this method, since the peptide synthesized remains in solution, it can be isolated, purified and its purity can be checked before use in the subsequent reactions in the step-wise method. This is in contrast to the Merrifield method where the peptide is cleaved from the support only after the entire sequence of the synthetic steps is completed. Thus the possibility of isolation and purification of the peptides formed at each stage eliminates the heterogeneity of the final product in the polymeric reagent method. The coupling efficiency in this method can also be increased by using excess of the polymeric active ester; the excess reagent after coupling can be removed by filtration. The polymeric reagent method has been applied successfully to the syntheses of a number of small and medium-sized peptides (Fridkin *et al* 1977; Stern *et al* 1981), including cyclic peptides (Fridkin *et al* 1965; Patchornik *et al* 1967), in very pure form. The method offers the possibility of mechanization and automation (Wieland and Birr 1967; Stern *et al* 1981).

Polymer-bound mixed carboxylic dithiocarbamic anhydrides have been developed recently in our laboratory as reagents for the selective acylation of amino groups (Haridasan *et al* 1987; Haridasan and Pillai 1988). These reagents were found to activate the carboxyl component for the formation of the amide or peptide bonds. In this paper we report the preparation of a number of different polymeric acylating reagents of this type and their use in the synthesis of amides and some model peptides.

2. Experimental

2.1 *General*

Solvents used were reagent grade and were purified according to literature procedures. Microanalyses were performed by the Regional Sophisticated Instrumentation Centre, Lucknow. Melting points were determined on a hot-stage melting point apparatus and are uncorrected. Thin-layer chromatography was performed on pre-coated silica gel plates.

2.2 *Styrene-tetraethyleneglycol diacrylate copolymer (1)*

The monomers were washed with sodium hydroxide solution (1%, twice) and with water (thrice) to remove the inhibitor. Styrene (36·3 ml) and tetraethyleneglycol diacrylate (4·5 ml) were dissolved in a mixture of chloroform and methanol (1:1, 25 ml). Benzoyl peroxide (0·15 g) was added and the mixture heated for 4 h. The polymer obtained was filtered, washed with hot chloroform (30 ml, twice) and hot benzene (30 ml, twice) to remove all the soluble impurities. Finally the resin was washed with hot methanol (30 ml, thrice) and dried in an oven at 70°C. Yield 25 g. IR (KBr): 1740 (C=O, ester), 1120 (C–O–C), 1200–1210 (–C–C–O and 1060 (O–C–C) 695 and 760 cm^{-1} (aromatic).

2.3 *Aminofunctionalization of styrene-tetraethyleneglycol diacrylate copolymer*

2.3a *Friedel-Crafts reaction with N-chloromethyl phthalimide – Preparation of phthalimidomethyl resin* (2): Crosslinked polystyrene (1, 10 g) was allowed to swell in dichloromethane (50 ml) and kept at 0°C. N-chloromethyl phthalimide (10 g, 100 mmol) was dissolved in CH_2Cl_2 (20 ml) and cooled in an ice-bath. Anhydrous $AlCl_3$ (13·2 g, 100 mmol) was added to this solution slowly with frequent shaking. The resulting complex was added in small portions to the swollen resin with stirring. The mixture was stirred at 0°C for 30 min and then at room temperature for 24 h. The reaction mixture was filtered, washed with dichloromethane, dioxane, dioxane–water (1:1), dioxane–2N HCl (1:1), water, ethanol and finally with methanol, drained and dried under vacuo to afford phthalimidomethyl resin (2). IR (KBr): 1780, 1710 cm^{-1} (C=O).

2.3b *Hydrazinolysis of phthalimidomethyl resin to aminomethyl resin* (3): A mixture of phthalimidomethyl resin (10 g) and a solution of hydrazine hydrate (95%, 8 ml) in ethanol (200 ml) was heated under reflux for 5 h. The resin was filtered, washed with DMF, DMF–water, water–dioxane, water, ethanol and finally with methanol, drained and dried under vacuo to afford aminomethyl resin (3). Amino group capacity of the resin was determined by the picric acid method (Gisin 1972) and found to have a capacity of 3·4 mmol NH_2/g.

2.4 *Preparation of sodium dithiocarbamate resin* (4)

The amino resin (10 g, 34 mmol) was added to a five-fold molar excess of an equimolar mixture of carbondisulphide (11 ml, 170 mmol) and sodium hydroxide (6·8 g, 170 mmol) and the reaction mixture shaken for 10 h. The resin was then collected by filtration and washed several times with water, ethanol, methanol and chloroform (50 ml, 5 times) and dried under vacuo to afford the dithiocarbamate resin (4). Yield = 13·2 g. IR (KBr): 3400 (NH), 1445 (C–N), 1345 and 1270 (C–N), 1210 (NCS_2), 1005 (C=S) and 1740 cm^{-1} (C=O).

2.5 *Polymer-bound mixed benzoic dithiocarbamic anhydride* (5a)

The dithiocarbamate resin (4, 10 g) was suspended in a mixture of acetonitrile and chloroform (1:1, 50 ml). Benzoyl chloride (10 ml) was added to the suspension and the mixture shaken for 4 h. The reaction mixture was filtered to collect the resin, washed with chloroform, acetonitrile, water, ethanol and finally with methanol, drained and dried under vacuo. Yield 10·6 g, IR (KBr): 3400 (NH), 1700 (C=O), 1450 (C=N), 1275 (C–N), 1205 (NCS_2) and 1010 cm^{-1} (C=S).

2.6 *Benzoylation of amines using resin 5a*

A suspension of the anhydride resin (5a, 1 g), aniline (0·5 mmol) and chloroform (20 ml) was shaken for 2 h. The mixture was then filtered and washed with chloroform (15 ml, thrice). The filtrate, together with the washings, was collected, washed with HCl (1N, 10 ml) to remove any unreacted aniline followed by washing with water

Table 1. Benzoylation of amines using polymer-bound mixed benzoic dithiocarbamic anhydride (5a).

Amine	Solvent	Duration (h)	Yield (%)	m.p. (°C)
Aniline	$CHCl_3$	2·0	95	163
p-Chloroaniline	$CHCl_3$	2·5	94	183
m-Toluidine	$CHCl_3$	2·0	90	118
p-Toluidine	$CHCl_3$	2·5	85	155
m-Aminophenol	$CHCl_3$	2·5	90	158
Methylamine	Dioxane	5·0	80	82
Glycine ethyl ester	THF/H_2O (1:1)	4·0	65	82
Glycine	Dioxane/water (1:1)	5·0	60	186

and dried over anhydrous $CaCl_2$. Evaporation of the solvent afforded benzanilide. Yield 90 mg (95%), m.p. 162°C. Details of the products obtained are given in table 1.

2.7 *Polymer-bound p-nitrobenzoic dithiocarbamic anhydride (5b)*

A mixture of the dithiocarbamate resin (4, 10 g), acetonitrile (30 ml), chloroform (30 ml) and *p*-nitrobenzoyl chloride (10 g) was shaken for 4 h. The product resin was collected by filtration and washed with acetonitrile, water, ethanol and methanol (25 ml, thrice) and dried in vacuum. Yield = 10·5 g, IR (KBr): 3400 (NH), 1350, 1530 (NO_2), 1710 (C=O), 1450 (C=N), 1370, 1275 (C–N), 1205 (NCS_2) and 1005 cm^{-1} (C=S).

2.8 *p-Nitrobenzoylation of amines using resin 5b*

In a typical reaction, a mixture of the resin 5b (1 g), aniline (0·05 g, 0·5 mmol) and chloroform (20 ml) was shaken for 2 h. The residual resin was filtered and washed with chloroform (15 ml, twice). The filtrate, together with the washings, was treated with HCl (1N, 10 ml), to remove the unreacted aniline and then with water (25 ml). After drying over anhydrous $CaCl_2$, the solvent was removed by evaporation to get the amide. Yield: 110 mg (90%), m.p. 210°C. Details of the amides prepared with the resin are given in table 2.

2.9 *Polymer-bound mixed acetic dithiocarbamic anhydride (5c)*

A mixture of the resin (10 g), acetonitrile (30 ml) and chloroform (30 ml) was cooled in an ice-bath. Acetyl chloride (10 ml) was added to this mixture with constant stirring. After 30 min the mixture was shaken for 4 h. The resin was collected by filtration and washed successively with acetonitrile, water, ethanol and methanol (25 ml, thrice) and dried in a vacuum desiccator. Yield: 10·2 g. IR (KBr): 3400 (NH), 1700 (C=O), 1440 (C=N), 1370, 1275 (C–N), 1205 NCS_2) and 1010 cm^{-1} (C=S).

2.10 *Acetylation of amines using resin 5c*

A mixture of the resin 5c (1 g), methylamine (0·5 ml of 35% solution) and chloroform (20 ml) was shaken for 5 h. The residual resin was separated by filtration and washed with

Table 2. *p*-Nitrobenzoylation of amines using polymer-bound mixed *p*-nitrobenzoic dithiocarbamic anhydrides (5b).

Amine	Solvent	Duration (h)	Yield (%)	m.p. (°C)
Aniline	$CHCl_3$	2·0	90	210
p-Chloroaniline	$CHCl_3$	2·0	85	190
m-Toluidine	$CHCl_3$	2·5	83	143
p-Toluidine	$CHCl_3$	2·5	85	198
m-Aminophenol	$CHCl_3$	2·5	82	201
Methylamine	Dioxane	4·0	60	218
Glycine	Dioxane/water (1:1)	4·0	60	129

Table 3. Acetylation of amines using polymer-bound mixed acetic dithiocarbamic anhydride (5c).

Amine	Solvent	Duration (h)	Yield (%)	m.p. (°C)
Aniline	$CHCl_3$	3·0	75	112
p-Chloroaniline	$CHCl_3$	3·0	67	172
m-Toluidine	$CHCl_3$	3·5	63	58
p-Toluidine	$CHCl_3$	3·5	65	153
m-Aminophenol	$CHCl_3$	3·5	60	148
Methylamine	Dioxane	5·0	60	82
Glycine	Dioxane/water (1:1)	5·0	62	204

chloroform (15 ml, twice). The filtrate, together with the washings, was treated with HCl (1N, 10 ml) to remove the unreacted amine and then with water (25 ml). After drying with anhydrous $CaCl_2$, the solvent was removed by evaporation to get the product. Yield: 35 mg, m.p. 82°C. Details of the reaction of the resin 5c with other amines are given in table 3.

2.11 *Polymer-bound BzGly* (7)

A mixture of Bz–Gly (2 g) and thionyl chloride (6 ml) was shaken for 1 h. The excess thionyl chloride was removed by distillation under reduced pressure and the residue boiled with charcoal and benzene (25 ml) and filtered to get benzoylglycyl chloride. The dithiocarbamate resin 4 (2 g) was then added to the solution of Bz–Gly–OCl and the resulting mixture was shaken for 6 h. The product resin was filtered, washed and dried to afford the resin 7. Yield 2·075 g. IR (KBr): 3400 (NH), 1700 (C=O), 1445 (C=N), 1345 and 1270 (C–N), 1210 (NCS_2) and 1005 cm^{-1} (C=S).

2.12 *Synthesis of the peptide Bz–Gly–Gly–OEt*

Glycine ethyl ester hydrochloride (0·14 g, 1 mmol) was suspended in THF (20 ml) and neutralized with triethylamine. The mixed N-protected amino acyl dithiocarbamic anhydride resin (1 g) was added to this solution and the mixture shaken for 6 h. The

residual resin was removed by filtration and washed with THF. The combined filtrate and washings were evaporated. The residue was extracted with dichloromethane and washed with citric acid solution (10%, 10 ml × 2 min, twice) and water. After drying over anhydrous $CaCl_2$, the solvent was removed by evaporation to afford the dipeptide Bz–Gly–Gly–OEt. Yield: 60 mg (46%), m.p. 90°C. IR (KBr): 3400 (NH), 1690 (C=O, amide) 1750 cm^{-1} (C=O, ester) R_f^a: 0·46 (*a* – in a mixture of chloroform: methanol = 9:1). Analysis, calculated for $C_{13}H_{16}N_2O_4$; C, 59·09; H, 6·06; N, 10·60%; found: C, 58·42; H, 5·91; N, 10·25%.

2.13 *Synthesis of the peptide Bz–Gly–Phe–OMe*

Phenylalanine methyl ester hydrochloride (0·107 g, 0·5 mmol) was suspended in dichloromethane (20 ml) and neutralized with triethylamine. The Bz–Gly resin (7, 0·5 g) was added to this solution and the mixture shaken for 6 h. The residual resin was filtered, washed with CH_2Cl_2 (10 ml, twice). The combined filtrate and washings were washed with citric acid solution (10%, 10 ml, twice), and water. The organic layer was separated and dried over anyhydrous $CaCl_2$ and the solvent was removed by evaporation to afford the peptide Bz–Gly–Phe–OMe. Yield: 100 mg (63%), m.p. 112°C. R_f^a: 0·51. IR (KBr): 3400 (NH), 1690 (C=O, amide), 1740 (C=O, ester). Analysis, calculated for $C_{19}H_{20}N_2O_4$: C, 67·06; H, 5·88; N, 8·23%; found: C, 66·81; H, 5·72; N, 8·01%.

2.14 *Reaction of resin 4 with Boc–Leu–anhydride. Preparation of resin 8a*

Boc–Leu (0·92 g, 4 mmol) was dissolved in dichloromethane (20 ml), and treated with DCC (0·412 g, 2 mmol) at 0°C. The precipitated dicyclohexylurea was removed by filtreation. Dithiocarbamate resin (0·5 mg) and pyridine (1·5 ml) were added to the filtrate. The mixture was stirred for 10 h. After reaction, the resin was collected by filtration, washed with dichloromethane (10 ml, 5 times), THF (10 ml, twice), drained and dried under vacuo. Yield: 0·55 g. IR (KBr): 1715 (C=O), urethane, 3400 (NH), 1445 (C=N), 1345 and 1270 (C–N), 1210 (NCS_2) and 1010 cm^{-1} (C=S).

2.15 *Reaction of the Boc–Leu–resin with Phe–OMe. Preparation of Boc–Leu–Phe–OMe*

Phenylalanine methyl ester hydrochloride (0·11 g, 0·5 mmol) was suspended in dichloromethane (20 ml) and neutralized with triethylamine. Boc–Leu–anhydride resin (0·5 g) was added to this solution and the reaction mixture stirred for 10 h. The residual resin was filtered and washed with dichloromethane (15 ml, twice). The filtrate, together with the washings, was treated with citric acid solution (10%, 10 ml, twice) and with water. After drying over anhydrous $CaCl_2$, the solvent was removed by evaporation to obtain the peptide Boc–Leu–Phe–OMe. Yield: 70 mg (48%), m.p. 85°C. R_f^a = 0·42. IR (KBr): 1650 (amide); 1710 cm^{-1} (urethane). NMR ($CDCl_3$): δ 1·45 (*s*, 9H, Boc), 0·95 (*d*, 6H, $C_\delta H$, Leu); 1·7 (*m*, 3H, C_γ, $C_\beta H$, Leu): 3,75 (*s*, 3H, OCH_3, Phe): 4·1 (*q*, 1H, $C_\alpha H$, Phe), 4·4 (*q*, 1H $C_\alpha H$, Leu) 6·2 (*d*, 1H, NH, Phe); 6·5 (*d*, 1H, NH, Leu); 7·25 (*s*, 5H aromatic, Phe). Analysis, calculated for $C_{21}H_{32}N_2O_5$: C, 63·16; H, 8·45; N, 7·37%; found: C, 62·61; H, 8·19, N, 7·18%.

2.16 *Boc–Gly–resin 8b*

Boc–Gly (0·35 g, 2 mmol) was dissolved in dichloromethane (20 ml) and DCC (0·206 g, 1 mmol) added to this solution. The mixture was stirred at 0°C for 10 min and at room temperature for 30 min. The precipitated dicyclohexylurea was removed by filtration. To the filtrate the dithiocarbamate resin (0·5 g) and pyridine (1 ml) were added. The reaction mixture was stirred for 10 h. The resin was collected by filtration, washed with dichloromethane, drained, and dried under vacuo. Yield: 0·54 g.

2.17 *Synthesis of Boc–Gly–Phe–OMe*

Phenylalanine methyl ester hydrochloride (0·108 g, 0·5 mmol) was neutralized with triethylamine in dichloromethane (20 ml). Boc–Gly–anhydride resin (0·5 g) was added to this solution and the mixture stirred for 10 h. The spent resin was filtered and washed with dichloromethane. The filtrate combined with the washings was treated with citric acid solution (10%, 10 ml, twice) and water. After drying over anhydrous $CaCl_2$ the solvent was evaporated to afford the peptide Boc–Gly–Phe–OMe. Yield: 100 mg, m.p. 112°C. R_f^a: 0·36. Analysis, calculated for $C_{17}H_{24}N_2O_5$: C, 60·71; H, 7·14; N, 8·33%; found: C, 60·25; H, 7·01; N, 8·18%.

2.18 *Synthesis of Boc–Leu–Met–NH_2*

Methionineamide hydrochloride (0·092 g, 0·5 mmol) was suspended in tetrahydrofuran (20 ml) and neutralized with triethylamine (0·5 ml). Boc–Leu–mixed dithiocarbamic anhydride resin (0·5 g) and pyridine (1 ml) were added to this solution and the reaction mixture stirred for 15 h. After the reaction, the spent resin was removed by filtration, washed with THF (10 ml, twice) and dichloromethane (10 ml, twice). The filtrate together with the washings was evaporated and the residue extracted with ethyl acetate (30 ml) and treated with citric acid solution (10%, 10 ml, twice) and water. After drying over anhydrous $CaCl_2$, the solvent was removed by evaporation to afford the dipeptide Boc–Leu–Met–NH_2. Yield: 110 mg, m.p. 156°C. R_f^a: 0·46. IR (KBr): 3450–3400 (NH_2), 1710 cm^{-1} (urethane). Analysis, calculated for $C_{16}H_{31}SN_3O_4$: C, 53·18; H, 8·59, N, 11·63%; found: C, 52·85, H, 8·40; N, 11·28%.

2.19 *Deprotection of Boc–Leu–Met–NH_2*

Boc–Leu–Met–NH_2 (110 mg) was treated with HCl–HOAc (5 ml) for 0·5 h at room temperature. The solvent was removed by vacuum evaporation and the hydrochloride salt of the dipeptide precipitated by addition of dry ether. The completion of the reaction was checked by TLC. Yield: 90 mg.

2.20 *Synthesis of Boc–Gly–Leu–Met–NH_2*

Leu–Met–NH_2·HCl (0·09 g, 0·3 mmol) was suspended in THF (20 ml) and neutralised with triethylamine. To this solution was added the resin 8b (0·5 g) and the mixture was stirred for 24 h. The residual resin was separated by filtration, washed with THF (10 ml, twice), dichloromethane (10 ml, twice) and ethyl acetate (10 ml, twice). The

combined filtrate and washings were evaporated. The residue was extracted with ethyl acetate, treated with citric acid solution (10%, 10 ml, twice) and then water, and dried over anhydrous $CaCl_2$. The solvent was evaporated to obtain the tripeptide Boc–Gly–Leu–Met–NH_2. Yield: 80 mg, m.p. 115°C, R_f^a: 0·36. IR (KBr): 3450–3400 (NH), 1710 cm^{-1} (urethane). Analysis, calculated for $C_{18}H_{34}SN_4O_5$: C, 51·67; H, 8·13; N, 13·4%; found: C, 51·15; H, 7·85, N, 12·9%. Amino acid analysis: Gly, 0·98 (1), Leu, 0·99 (1), Met, 1·15 (1).

2.21 *Preparation of Z–Phe–resin 8c*

Z-phenylalanine (0·57 g, 2 mmol) was dissolved in dichloromethane (20 ml) and dicyclohexylcarbodiimide (0·205 g 1 mmol) was added. The mixture was stirred at 0°C for 10 min and then at room temperature for 30 min. The precipitated dicyclohexylurea was removed by filtration. To this solution was added the sodium dithiocarbamate resin (4, 0·5 g) and pyridine (1·5 ml). The reaction mixture was stirred for 15 h. The resin was collected by filtration, washed with dichloromethane several times, drained and dried under vacuo. Yield 0·55 g.

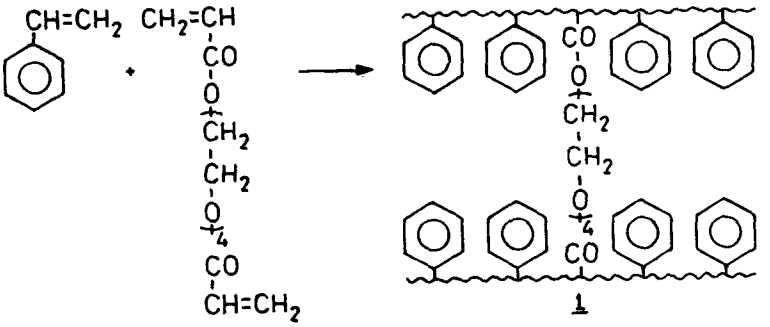
2.22 *Synthesis of the peptide Z–Phe–Phe–OMe*

Phenylalanine methyl ester hydrochloride (0·108 g, 0·5 mmol) was suspended in dichloromethane and neutralized with triethylamine. Z–Phe–dithiocarbamic anhydride resin (8c, 0·5 g) was added to this solution. The mixture was stirred for 15 h. The residual resin was separated by filtration and washed with dichloromethane. The filtrate and combined washings were treated with citric acid solution (10%, 10 ml, thrice), water and dried over anhydrous $CaCl_2$. The solvent was removed by evaporation to afford the peptide Z–Phe–Phe–OMe. Yield: 40 mg (40%). m.p. 135°C, $R_f^a = 0·5$. Analysis, calculated for $C_{26}H_{26}N_2O_5$; C, 69·95; H, 5·83; N, 6·28%; found: C, 69·34; H, 5·72; N, 6·08%.

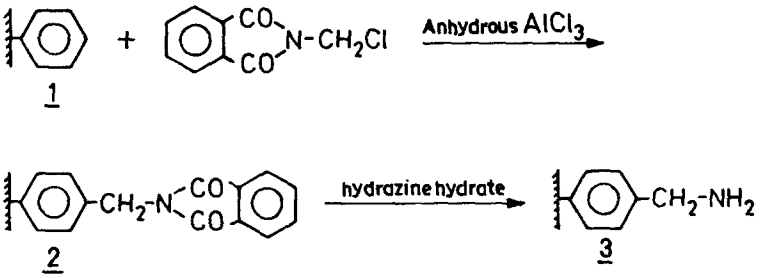
3. Results and discussion

3.1 *Preparation and characterization of polymer-bound mixed carboxylic dithiocarbamic anhydrides*

Crosslinked polystyrene was selected as the macromolecular support, in the present investigation, on account of its ease of preparation and of incorporation of various functional groups, and its good mechanical characteristics (Frechet and Farral 1977). Polystyrenes crosslinked with hydrophobic divinylbenzene and hydrophilic tetraethyleneglycol diacrylate were used as the supports, while tetraethyleneglycol diacrylate crosslined supports were found to be more suitable for the preparation of the polymeric reagents. The polymer support (1) was prepared by copolymerizing styrene with 5 mol% tetraethyleneglycol diacrylate (scheme 1). The resin 1 was converted to aminomethyl polystyrene (3) by a two-step polymer-analogous reaction. Friedel-Crafts reaction of resin 1 with N-chloromethyl phthalimide in dichloromethane to phthalimidomethyl resin (2) and subsequent hydrazinolysis afforded aminomethyl polystyrene (3) (scheme 2).

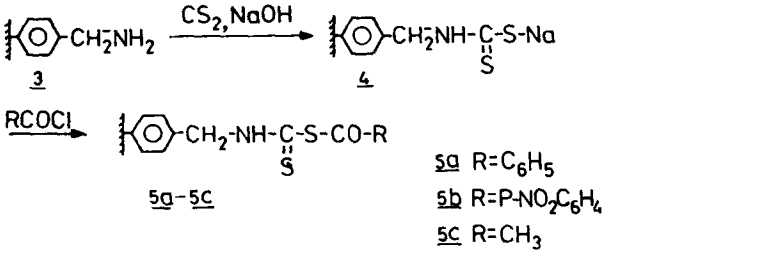


Scheme 1. Preparation of tetraethyleneglycol-crosslinked polystyrene.



Scheme 2. Preparation of aminomethyl polystyrene.

The capacity of the aminomethyl resin was determined by spectrophotometric methods (Gisin 1972) using picric acid. The amino resin was found to have a capacity of 3·4 mmol amino groups per gram of the resin. Treatment of the aminomethyl resin with a five-fold molar excess (based on the amino group capacity) of an equimolar mixture of carbondisulphide and sodium hydroxide in aqueous solution gave the sodium dithiocarbamate resin (4). The completion of the reaction was tested by semiquantitative ninhydrin reaction (Spackman *et al* 1958). The dithiocarbamate resin (4) on treatment with benzoyl, *p*-nitrobenzoyl and acetyl chlorides gave the corresponding polymer-bound mixed carboxylic dithiocarbamic anhydride resins (5a)–(5c). The series of reactions is depicted in scheme 3.



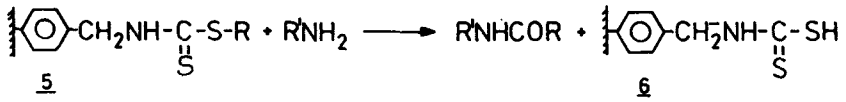
Scheme 3. Synthesis of polymer-bound mixed carboxylic dithiocarbamic anhydrides.

The IR spectrum of the dithiocarbamate resin 4 showed characteristic peaks at 3400 (NH), 1445 (C=N), 1345 and 1270 (CN), 1210 (NCS_2) and 1005 cm^{-1} (C=S). The conversion of resin 4 to 5 could be followed by the appearance of an absorption peak

around 1700 cm^{-1}, characteristic of the carbonyl group. The loading of the acyl group was determined by treating the anhydride resin with excess aniline and determining the weight of the benzanilide obtained.

3.2 *Synthesis of amides*

The mixed carboxylic dithiocarbamic anhydride resins (5a)–(5c) reacted with amines and amino acids dissolved in dioxane, chloroform or tetrahydrofuran, transferring their acyl groups to form the corresponding amides in solution and the dithiocarbamic acid resins (6) as the by-products (scheme 4). The product was separated by filtering the residual resin and washing with a suitable solvent. Evaporation of the solvent from the combined filtrate and washings afforded the amide.



Scheme 4. Acylation of amines using the polymeric reagent.

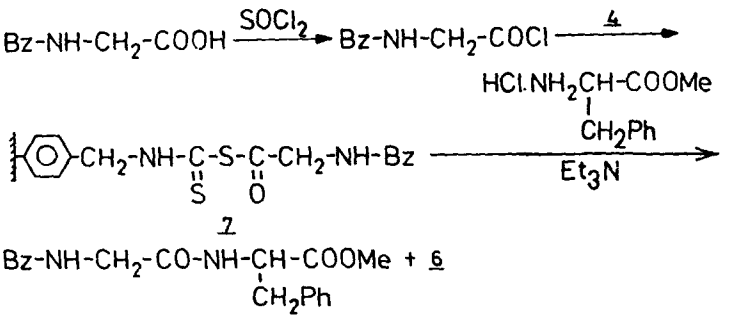
Using the dithiocarbamic anhydride resins (5a)–(5c), a number of amines were acylated. In the acylation reactions, the yields were in the range 60–95%. The reaction period varied from 2–5 h. Aromatic amines gave better yields than aliphatic amines. The products were characterized by melting points and by comparing with authentic samples.

3.3 *Synthesis of model peptides*

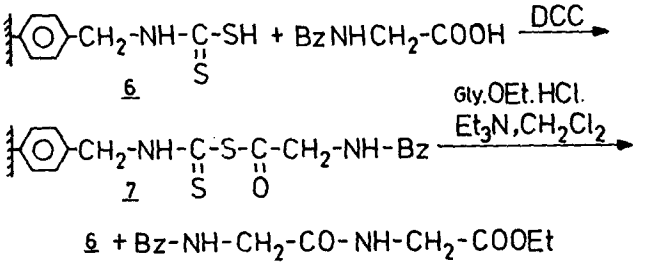
A few model peptides were synthesized using polymer-bound mixed amino acyl dithiocarbamic anhydrides. One of the disadvantages of these polymeric acylating reagents in peptide synthesis is that the synthesis of the reagent involves treatment with the acid chloride, the acyl group of which is to be transferred and hence this method cannot be applied to acid-sensitive amino acids or acid-cleavable protecting groups. The method can be applied however to photolytically cleavable N-protecting groups like N-2-nitrobenzyloxycarbonyl amino acids (Haridasan and Pillai 1987) and for N-benzoyl and N-acetyl amino acids. Benzoylglycine was converted to the corresponding acid chloride by treating with thionyl chloride. This amino acid chloride on treatment with resin 4 gave the corresponding mixed N-protected amino acyl dithiocarbamic anhydride resin 7. The resin 7 on treatment with a solution of phenylalanine methyl ester in dichloromethane gave the corresponding peptide Bz–Gly–Gly–OEt (scheme 5). Similarly the resin 7 on treatment with glycine ethyl ester afforded the dipeptide Bz–Gly–Gly–OEt.

Another method for the incorporation of amino acid residues onto the dithiocarbamate resin was attempted. The resin 4 was converted to the corresponding dithiocarbamic acid resin 6 by treating with dilute hydrochloric acid. The resin 6 was then coupled with N-benzoylglycine in the presence of DCC to form resin 7. This was then treated with a C-protected amino acid to afford the corresponding peptide. The synthesis of the peptide Bz–Gly–Gly–OEt by this method is depicted in scheme 6.

However, the degree of loading of the amino acyl group was found to be very low. The low degree of loading is attributed to the relative instability of the dithiocarbamic



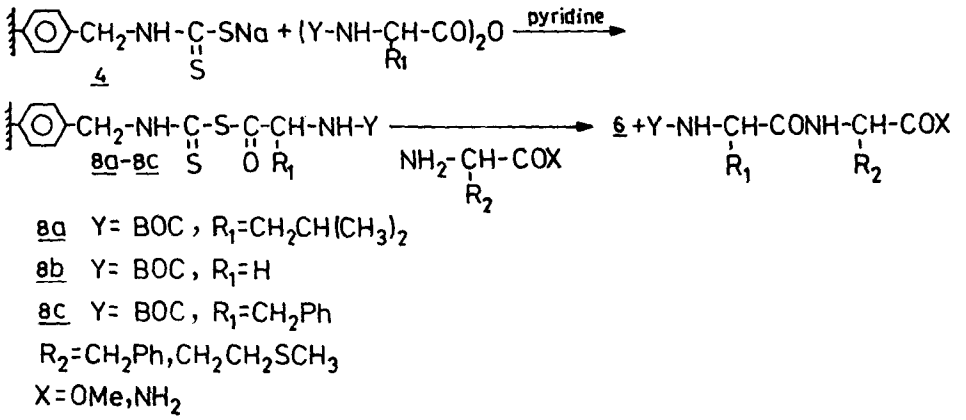
Scheme 5. Synthesis of peptides by the polymeric amino acylating agent prepared by the acid chloride method.



Scheme 6. Synthesis of the dipeptide Bz–Gly–Gly–OEt by polymeric reagent prepared by DCC coupling.

acid resin which is prone to decomposition forming the aminomethyl resin and carbondisulphide (Karchmer 1972). Moreover, the removal of the dicyclohexylurea precipitated inside the pores of the polymer matrix was difficult.

In another method for the incorporation of amino acid residues in the dithiocarbamate resin, the symmetric anhydride of the N-protected amino acid was prepared by treating with 0·5 equivalent of dicyclohexylcarbodiimide. The dicyclohexylurea precipitated was removed by filtration and the filtrate containing the anhydride was stirred with the resin 4 with equivalent amount of pyridine for 15 h. A two-fold molar excess of the anhydride was employed for acylating the resin. Synthesis of peptides by this method is given in scheme 7.



Scheme 7. Synthesis of peptides by the polymeric acylating agent prepared by the anhydride method.

Table 4. Peptides synthesized by polymer-bound mixed amino acyl dithiocarbamic anhydrides.

Peptide	Method	Solvent	Time (h)	m.p. (°C)	R_f^a	Yield (%)	Elemental analysis (%)* C	H	N
Bz–Gly–Gly–OEt	Acid chloride	CH_2Cl_2	4	98	0·46	46	58·42 (59·09)	5·91 (6·06)	10·25 (10·60)
							$C_{13}H_{16}N_2O_4$		
Bz–Gly–Phe–OMe	Acid chloride	CH_2Cl_2	4	125	0·51	50	66·81 (67·06)	5·72 (5·88)	8·01 (8·23)
							$C_{19}H_{20}N_2O_4$		
Z–Phe–Phe–OMe	Anhydride	CH_2Cl_2	15	135	0·50	40	69·34 (69·95)	5·72 (5·83)	6·08 (6·28)
							$C_{26}H_{26}N_2O_5$		
Boc–Gly–Phe–OMe	Anhydride	CH_2Cl_2	10	112	0·36	63	60·25 (60·71)	7·01 (7·14)	8·18 (8·33)
							$C_{17}H_{24}N_2O_5$		
Boc–Leu–Phe–OMe	Anhydride	CH_2Cl_2	10	85	0·42	48	62·61 (63·15)	8·19 (8·42)	7·18 (7·37)
							$C_{21}H_{32}N_2O_5$		
Boc–Leu–Met–NH_2	Anhydride	THF	15	156	0·35	45	52·85 (53·18)	8·40 (8·59)	11·28 (11·63)
							$C_{16}H_{32}N_3O_4S$		
Boc–Gly–Leu–Met–NH_2	Anhydride	THF	24	140	0·31	42	51·15 (51·67)	7·85 (8·13)	12·90 (13·40)
							$C_{18}H_{34}N_4O_5S$		

[a] Chloroform–mehanol mixture (9:1); * calculated values (in parenthesis) and molecular formulae are also shown

The dithiocarbamic anhydride resin prepared by this method exhibited similar characteristics as that prepared by the acid chloride method. The degree of acyl group loading in this method was relatively higher (1 mmol/g). Since this method does not involve the use of acidic reagents, this can be used for preparing the reagent with all amino acids. Some model peptides were synthesized using these amino acyl dithiocarbamic anhydrides.

In a model reaction, a C-protected amino acid was dissolved in dichloromethane or THF and treated with a two-fold molar excess of the polymeric acylating agent. The reaction mixture was stirred for 15–24 h. The reaction period varied according to the peptide synthesized. After the reaction, the spent resin was removed by filtration. The filtrate on evaporation afforded the peptide. The peptides were characterized by elemental analysis, IR and NMR spectroscopy and comparing with samples prepared by conventional methods. Details of the peptides synthesized are given in table 4.

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