



Solid Phase Synthesis of Cadaverine on Polymer Support

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Cadaverine is a naturally occurring polyamine which is closely associated with spermine and spermidine, two major polyamines which regulate growth processes in living beings. Using polystyrene as solid support and with the help of a photocleavable linker, cadaverine was synthesised. The only purification process involved throughout the synthesis is filtration and washing with suitable solvents. Cadaverine was first synthesised over polystyrene support and then cleaved from matrix photolytically. The yield and purity of the sample was studied using differently cross linked polystyrene resin with divinyl benzene as the cross linking agent. The effect of one photocleavable linker was studied. The synthesis of linker was done by classical organic synthetic procedure.

Key Words: Solid phase organic synthesis, Cadaverine, Linkers, Photolysis.

INTRODUCTION

Solid phase synthesis is a methodology whereby synthetic transformations are conducted with one of the reactant molecules attached to an insoluble material referred to as the solid support. It was originally developed for peptide synthesis. Since the recent impact of combinatorial chemistry, solid phase techniques have been applied more generally to organic synthesis. One of the requirements of solid phase chemistry is a linker to attach a substrate molecule to the solid phase. As synthesis proceeds, this material is transformed to the product, which can be finally removed by cleavage of the linker.

All polyamines especially cadaverine is intimately involved in normal growth processes¹⁻³. Cadaverine and their conjugates play an important role in the proliferations, differentiation and growth cycles of prokaryotic and eukaryotic⁴ cells. Research on polyamines has been the subject of numerous monographs⁵⁻⁷.

The present work of synthesis of cadaverine involves the synthesis of 1, 2 and 4 % crosslinked polystyrene matrix using divinyl benzene as the crosslinking agent. They are functionalized to get chloromethylated polystyrene resin (Merrifield resin). Merrifield resin was first nitrated and then subjected to Gabriel's phthalimide synthesis to get 3-nitro-4-aminomethyl polystyrene. Using these resins, synthesis of cadaverine was done.

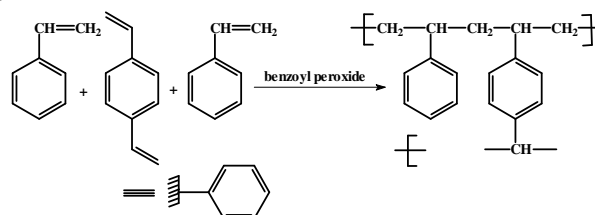
3-Nitro-4-aminomethyl polystyrene is an *o*-nitrobenzyl linker which is photocleavable which was attached to differently crosslinked polystyrene matrix and treated with appropriate reagents to obtain polystyrene supported cada-

verine. Finally, cadaverine was cleaved from the support by photolysis.

Synthesis of divinyl benzene crosslinked polystyrene with variable degree of crosslinking (A): Polystyrene-divinyl benzene (P.S.-DVB) copolymer supports of different degrees of crosslinking were prepared. The monomers, styrene and divinyl benzene were made free from inhibitors and were used for polymerization. Calculated amounts of the monomers mixed with the initiator, benzoyl peroxide were added to water, kept stirring at 80 °C containing poly(vinyl pyrrolidone) as the suspension stabilizing agent. The details of the reaction are presented in Table-1 and reaction sequence is presented in **Scheme-I**.

TABLE-1
YIELD AND CROSSLINK DENSITY OF
DVB CROSSLINKED POLYSTYRENE

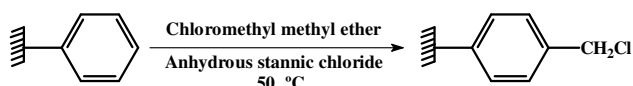
Crosslink density (mol %)	Volume of monomers		Yield (%)
	Styrene (mL)	DVB (mL)	
1	18.7	0.45	88
2	18.7	0.90	90
4	18.7	1.80	91



Scheme-I: Synthesis of divinyl benzene crosslinked polystyrene (A)

Preparation of chloromethylated polystyrene (B):

Anhydrous zinc chloride and stannic chloride have been used in the chloromethylation of crosslinked polymers⁸. For chloromethylation, the adopted procedure⁹ was to add polystyrene beads (A), pre-swollen in dichloromethane to a well stirred suspension of anhydrous stannic chloride in chloromethyl methyl ether kept at 0 °C. Polystyrene resin was allowed to pre-swell in a good organic solvent was normally used since it was found to give better reaction efficiency. The product resin was dried under vacuum at 60 °C. The reaction is depicted in **Scheme-II**. The chlorine capacity was determined using modified Volhard's method. The chlorine capacity is tabulated in Table-2.



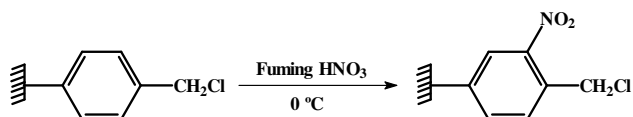
Scheme-II: Preparation of Chloromethylated polystyrene

Cross-link density (mol %)	Chlorine capacity (mmoles/g)	NH ₂ capacity (mmol/g)	IR (KBr) (cm ⁻¹)	
			Resin-C	Resin-I
1	2.48	2.21	1350	
2	2.18	1.98	1525	3520
4	1.92	1.80	(NO ₂ str)	(NH str)

Preparation of photocleavable linker: Photolysis offers a mild and potentially orthogonal method of cleavage that takes place under neutral conditions. Photocleavable protecting groups have been used widely in carbohydrate chemistry, nucleotide and peptide synthesis.

Preparation of 3-nitro-4-aminomethyl polystyrene (I):

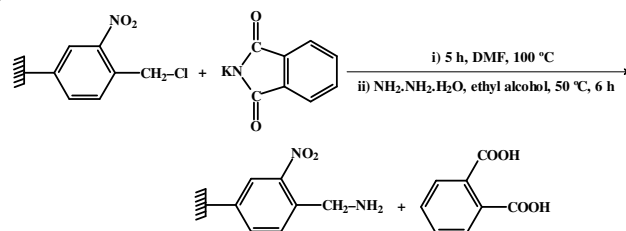
The first step in the preparation of 3-nitro-4-aminomethyl polystyrene (I) is the preparation of 3-nitro-4-chloromethyl polystyrene (C). Polystyrene could be nitrated easily using nitric acid¹⁰. It has been found to be a low temperature reaction and the optimum temperature was found to be 0- 5 °C. Fuming nitric acid of specific gravity 1.42 is enough and mono nitration occurs as was evident from the percentage of nitrogen content. The presence of nitro group was supported by elemental analysis. The presence of nitro group at 3-position of the phenyl ring was supported by IR spectra which showed strong absorption bands at 1525 and 1350 cm⁻¹. Analytical and spectral details are given in Table-2. The reaction is given in **Scheme-III**.



Scheme-III: Preparation of 3-nitro-4-chloromethyl polystyrene (C)

3-Nitro-4-aminomethyl polystyrene was prepared from 3-nitro-4-chloromethyl polystyrene by Gabriel's phthalimide synthesis¹¹ which involves two steps: 3-nitro-4-chloromethyl polystyrene, pre-swollen in DMF was reacted with potassium phthalimide. The phthalimidomethyl resin formed showed IR

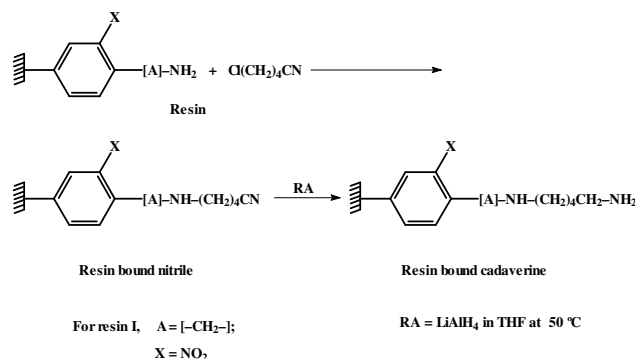
absorption due to C-N str. at 1380 cm⁻¹ and C=O str. at 1740 cm⁻¹. Phthalimidomethyl resin was subjected to hydrazinolysis. Resin in ethyl alcohol was mixed with hydrazine hydrate and heated under reflux. The presence of free NH₂ group was confirmed by ninhydrin test (**Scheme-IV**). The amino capacity of the resin was determined by titrimetric method. Spectral and analytical data are given in Table-2.



Scheme-IV: Preparation of 3-nitro-4-aminomethylpolystyrene resin (I)

Preparation of resin bound cadaverine using polystyrene bearing different photocleavable linkers:

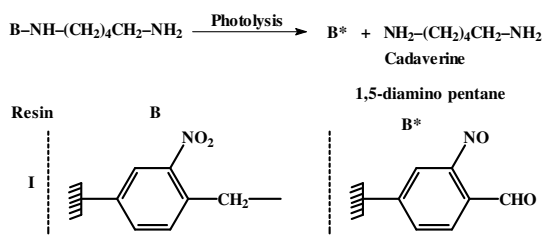
Cadaverine was synthesised using a photocleavable linker attached to differently crosslinked polystyrene resin. Differently crosslinked polystyrene containing photocleavable linker was reacted with chloropentanitrile in pentanol¹². IR spectrum of the resin bound nitrile showed $\nu(\text{C-N})$ at 2200 cm⁻¹. The resin bound nitrile suspended in THF was treated with appropriate reducing agent to obtain the resin bound cadaverine. The general reaction sequence is given in **Scheme-V**.



Scheme-V: Reaction sequence for the synthesis of polystyrene resin bound cadaverine

After reduction the resin gave ninhydrin test and the IR spectrum showed NH str at 3520 and 3480 cm⁻¹. The reaction sequence is given by **Scheme-V**. Addition of chloro pentanitrile over differently crosslinked resin bearing -NH₂ groups resulted in the formation of resin bound nitrile at very low temperature in presence of calculated amount of chloropentano nitrile, mono alkylation occurs.

The reduction of the monoalkylated product was done selectively to avoid the reduction of the chromophoric group present in the linkers. Chromophoric groups are introduced into the linker moiety for avoiding the destruction of the polyamine molecule. The chromophoric group selectively absorbs light and cleavage of synthetic polyamine from the support occurs. The reduction was done with lithium aluminium hydride in THF at 50 °C.



Scheme-VI: Photolysis of resin bound cadaverine

Photolysis of polystyrene bound cadaverine: Photolysis offers a mild and potentially orthogonal method of cleavage that takes place under neutral conditions for the release of the synthesized product from the solid support¹³. Synthesis was carried out effectively on solid supports in earlier days, but the cleavage was found to be difficult one. The smooth release of the product can be achieved by using photocleavable linkers. In case *o*-nitrobenzyl protecting groups, the nitro group is reduced to a nitroso function while one oxygen atom get inserted into the C-H bond located in the *ortho* position. The reaction appears to be general for all *ortho* substituted nitrobenzene derivatives having a hydrogen atom in the side chain.

Cadaverine was synthesised stepwise on an *o*-nitrobenzyl resin attached to polystyrene resin. The differently crosslinked polystyrene resin bound cadaverine was subjected to photolysis in a RPR 100 apparatus equipped with immersion type photometer. Wavelength below 320 nm was filtered out to avoid unwanted side reactions. Photolysis was continued for 12-36 h. The linker is photocleavable which release cadaverine without decomposing them and leaving behind the aldehyde polymer which remains insoluble and can be filtered out.

Using differently crosslinked resin bound cadaverine photolysis was carried out by suspending the resin in methanol. After irradiation the aldehyde polymer was filtered out and purified by chromatographic separation. The general scheme of photolysis of polystyrene bound cadaverine resin is given in Scheme-VI. The yield of cadaverine is presented on Table-3. The spectral and analytical data of cadaverine is presented in Table-4.

TABLE-3
EFFECT OF EXTENT OF CROSS LINKING
AND YIELD OF CADAVERINE

Yield (%) of cadaverine		
1 (%)	2 (%)	4 (%)
61.60	64.2	60.02

EXPERIMENTAL

The monomers, styrene and divinyl benzene were obtained from Sigma Aldrich Corporation, USA and were distilled under reduced pressure. All other chemicals of AR grade were

procured from local vendors. All solvents were purified by standard procedures. The IR spectra were recorded on a Perkin Elmer spectrum I spectrophotometer using KBr pellets at Madurai Kamaraj University. The NMR spectra were recorded on a Joel GSX 400 MHz FTNMR spectrometer at SAIF, IIT, Chennai. CHN analysis was done at RRL, Thiruvananthapuram.

Preparation of polystyrene-DVB support: The monomers, styrene and divinyl benzene were purified by low-pressure distillation. They were washed with sodium hydroxide (1 % solution) three times and water to remove inhibitors. The crosslinked polymers were prepared by suspension polymerization technique. Poly(vinyl pyrrolidone) (150 mg) was dissolved in distilled water (100 mL) and heated to 80 °C. The mixture of monomers, styrene (18.7 mL) and divinyl benzene (0.9 mL), for 2 % degree of crosslinking, was mixed with the initiator benzoyl peroxide (200 mg) and added slowly to the hot solution with constant stirring. The mixture was heated at 90 °C with continuous stirring for 12 h. The polymer formed during the process was collected by filtration and washed with hot water, ethanol, toluene, acetone and methanol and the beads were dried at 60 °C in vacuum. Polymers with different degree of crosslinking, *viz.* 1 and 4 % were prepared by varying the ratio of the monomers suitably.

Preparation of chloromethylated polystyrene: 2 % divinylbenzene crosslinked polystyrene (25 g) was swollen by stirring at 25 °C for 1 h in chloroform (150 mL) and cooled to 0 °C. A cold solution of anhydrous SnCl₄ (3.8 mL) in chloromethylmethyl ether (50 mL) was added and stirred for 5 h at 0 °C. The mixture was filtered and washed with (1 × 10 mL) of 3:1 dioxane-water and then with (1 × 10 mL) of 3:1 dioxane -3N HCl. The cream coloured beads were washed further with solvents which changed gradually from water to pure dioxane and then progressively to 100 % methanol. Abrupt changes of solvent compositions were avoided. The chlorine capacity of the product was determined which showed 2.18 m moles of Cl/g of polymer. Yield 30 g.

Preparation of 3-nitro-4-chloromethyl polystyrene: A sample of dry chloromethyl polymer (5 g, 2.18 m moles of Cl/g) was added slowly with stirring to 500 mL of fuming nitric acid (90 % HNO₃, sp. gr. 1.5) which was cooled to 0 °C. The mixture was stirred for 1 h at 0 °C and poured into crushed ice. The beads were filtered and washed with water, dioxane and methanol and dried. The nitrogen content was found to be 6.38 m moles/g by Kjeldahl method. This was equivalent to 1.03 nitro group per aromatic ring. Yield 6.6 g.

Estimation of chlorine capacity of chloromethylated polystyrene-Volhard's method: Chloromethylated polystyrene (0.3 g) of polymer was heated in pyridine (3 mL) at 100 °C for 2 h. This suspension was transferred to 250 mL conical flask containing 50 % acetic acid (30 mL). Conc. HNO₃ (5 mL) was added, followed by slow addition of AgNO₃

TABLE-4
SPECTRAL AND ANALYTICAL DETAILS OF CADAVERINE HYDROCHLORIDE

Elemental analysis (%) (KBr, ν_{\max} , cm ⁻¹)			IR	¹ HNMR	¹³ CNMR
Element	Calculated	Found			
C	58.8	58.75	NH _{str} , 3500	δ 1.4 -1.6 (t, 4H) δ 2.5-2.7 (t, 6H)	Three peaks δ 32, (2C), δ 28, (2C), δ 23, (1C)
H	13.72	13.62			
N	27.43	26.73			

(10 mL, 0.1 N), during magnetic stirring. The suspension was mixed well. The excess of AgNO_3 was back titrated with standard ammonium thiocyanate solution using ferric alum as indicator. A blank was also conducted. From the titre values the chlorine capacity of the polymer was determined.

Estimation of amino capacity of the differently cross-linked aminomethyl polystyrene resin: Hydrochloric acid (0.1 N, 40 mL) was added to polymer amine (200 mg). It was kept for 24 h. The resin was filtered, washed and the filtrate was titrated against NaOH using phenolphthalein as indicator. A blank was conducted with 0.1N HCl and NaOH. From the titre values amino capacity was determined and was found to be 1.98 m moles/g

Preparation of 3-nitro-4-aminomethyl polystyrene (I): The preparation of 3-nitro-4-aminomethyl polystyrene involved two steps: preparation of the phthalimidomethyl resin and the subsequent hydrazinolysis. 3-Nitro-4-chloromethyl polystyrene containing 2.18 m moles of chlorine per g was made use of. The resin (10 g) swollen in $\text{N,N}'$ -dimethyl formamide (100 mL) was mixed with potassium phthalimide (8.9 g) and was stirred for 6 h at 100 °C. Potassium phthalimide was prepared by grinding anhydrous potassium carbonate (10 g) and phthalimide (19 g) using glass mortar. Phthalimidomethyl resin was washed with distilled water (10 mL \times 3 mL) and methanol (10 mL \times 3 mL). Phthalimidomethyl resin was mixed with hydrazine hydrate (1 mL/g of the polymer) and ethanol (50 mL). It was heated under reflux for 6 h. The product was filtered and washed with water (10 mL), methanol (30 mL) and finally with dichloromethane (10 mL). Yield 8.82 g.

Preparation of differently crosslinked polystyrene resin bound cadaverine: Preparation of 3-nitro-4-aminomethyl polystyrene bound cadaverine was a two step process in which the first step was the formation of the nitrile, the second step was the formation of the dinitrile and the third step was the reduction of the nitrile to resin bound amine. The polymer (10 g, 2.2 m moles of NH_2/g of polymer) preswollen in THF, acrylonitrile (1.166 g, 22 m moles) was added dropwise and stirred for 12 h in water bath at 30 °C fitted with reflux condenser and guard tube. The product was kept for 2 h, filtered and washed successively with THF ether, ether-water and then dried. The IR spectrum of the resin showed $\text{C}\equiv\text{N}$ str. frequency at 2200 cm^{-1} confirming the presence of $\text{C}\equiv\text{N}$ group. The nitrile (10 g) was treated with chloro butyronitrile (1.14 g) in butanol (100 mL) in the presence of anhydrous sodium carbonate (3.37 g) and KI (3.71 g). The mixture was kept for 2 h and stirred for 12 h at 40 °C in water bath with reflux condenser and guard tube. It was allowed to stand for 6 h and the product was filtered and washed successively with THF ether-water, ether and finally with ethyl alcohol. Lithium aluminium hydride (0.083 g, 2.2 m moles) in anhydrous ether (10 mL) was added to resin bound mononitrile (10 g, 2.21 m moles of NH_2/g) in THF (10 mL). It was kept under reflux at 50 °C for 8 h using reflux condenser and guard tube. Excess lithiumaluminium hydride was removed by adding 30 % sodium hydroxide solution (50 mL). The product was washed with ether, ether-water mixture to obtain the resin bound cadaverine. The presence of free NH_2 group was confirmed by ninhydrin test. The IR spectrum showed NH str. frequency at 3520 cm^{-1} .

Photolysis of differently crosslinked polystyrene bound cadaverine: Polystyrene resin bound cadaverine (5 g) suspended in methanol (200 mL) was irradiated for 15 h in an RPR-100 apparatus equipped with immersion type photo reactor with wavelength 340 nm which released cadaverine and nitroso aldehyde polymer. The wavelength below 320 nm was filtered out by circulating 40 % CuSO_4 solution through the outer jacket. The nitroso aldehyde polymer was filtered out and its IR spectrum showed $\text{C}=\text{O}$ str frequency at 1700 cm^{-1} . The filtrate was collected quantitatively and purified by column chromatography. Filtrate was concentrated by evaporation and made to a paste by adsorbing on neutral alumina and eluted using ethyl acetate as the eluent. Distillation under vacuum gave a colourless liquid which was subjected to analysis.

RESULTS AND DISCUSSION

The use of polymer matrices as supports in organic synthesis continues to be an active field in solid phase synthesis since the pioneering work of Merrifield in the synthesis of a tetrapeptide for the first time using polystyrene support. Polymer supports and polymer supported reagents have become versatile because of its advantages like stability, easy work-up and selectivity.

A large number of new polymer supports were developed from polystyrene which were obtained by the polymerization of monomers using suitable crosslinking agents and properly functionalizing the polymers to suit the synthetic purpose. The proper choice of polymer support is an important step in solid phase organic synthesis. To make solid phase synthesis to be practical, several important issues need to be considered, including the correct choice of solid support, mode of attachment and cleavage of materials from the resin matrix. Efficiency in anchoring and removing a small organic molecule from the polymeric resin relies on the correct choice of the linker group also. This key fragment is crucial in planning a synthetic strategy.

Polystyrene is the most suitable support used for the synthesis of wide range of functionalities including amines. So, in present investigation the polymer support selected was divinyl benzene crosslinked polystyrene of different crosslink density which were prepared by varying the ratio of monomers, divinyl benzene and styrene. Using this polymer matrix as the support, the synthesis of cadaverine was done. Polystyrene was functionalised to get chloromethylated polystyrene (Merrifield resin). Preparation and attachment of photocleavable linker to the polystyrene matrix, their purification and characterisation, application of the photocleavable linker bearing resin in the synthesis of cadaverine, their purification and characterization were done by C, H, N analysis, ^1H NMR, ^{13}C NMR and mass spectrum.

Of the resins, 2 % crosslinked polystyrene gave maximum yield and minimum yield was noticed for 4 % crosslinked polystyrene resin. This is because 4 % crosslinked polystyrene was too rigid to permit easy penetration of reagents causing slower and lesser degree of functionalisation. The 1 % crosslinked resin beads were too fragile and disrupted during the photolysis and this made the filtration difficult. As filtration

being the main mode of purification, the difficulty in filtration might have led to low percentage yield of synthesized cadaverine while using 1 % crosslinked resin.

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