Synthesis, Characterization and Application of Tetraethylene Glycol Diacrylate Crosslinked Polystyrene Support for Gel Phase Peptide Synthesis

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SYNOPSIS

Styrene and tetraethylene glycol diacrylate (TTEGDA) were copolymerized by the free radical aqueous suspension polymerization technique, employing toluene as the monomer diluent at 80°C. The resulting beads were functionalized by chloromethylation. The copolymer was characterized by IR and high-resolution solid-state ^{13}C-NMR techniques. Scanning electron microscopy was employed to observe shape, size, and morphological features of the crosslinked bead copolymer. The swelling capacities of the copolymer were measured in various solvents. Reactivity of the amino functionalized polymer was compared with divinylbenzene-polystyrene (PS) resin. Stability of the copolymer was tested under various peptide synthetic conditions. High capacity chloromethyl TTEGDA-crosslinked PS was employed as a solid support in the synthesis of the hydrophobic peptide Boc(Ala-Leu-Ala)$_4$-OMe. The coupling and deprotection steps in the synthetic scheme proceeded in near quantitative yields, supporting the positive role of the flexible and hydrophilic crosslinking agent. The fully protected 12-residue peptide was cleaved from the support by a transesterification procedure in 85% overall yield and characterized by thin-layer chromatography, amino acid analysis, and ^{1}H-NMR. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Development of a polymeric support that swells in both polar and nonpolar solvents, facilitating the different types of organic reactions used in repetitive peptide synthesis, has been a challenge to polymer chemists ever since the introduction of solid phase peptide synthesis (SPPS) by Merrifield.[1] The Merrifield resin is basically nonpolar hydrophobic polystyrene (PS) crosslinked with rigid divinylbenzene (DVB) and functionalized with a chloromethyl group. This resin has been widely used in polymer supported reactions due to its commercial availability, mechanical stability, and ease of functionalization.[2] However, an extreme purity requirement and homogeneity of medium-to-large peptides is still a challenging problem in peptide synthesis using this support.[3,4] In the case of longer peptides, the rate of incorporation of a particular amino acid residue has been found to decrease with increasing chain length. The physicochemical incompatibility of the PS-DVB matrix with the attached peptide and the development of unfavorable conformational characteristics of the peptide and protein sequence are the factors responsible for the decrease in yield and purity in SPPS.[4]

Fridkin et al. used *O*-acyl polyhydroxy nitro PS as an acyl transferring agent in peptide synthesis.[5,6] In contrast to Merrifield's synthesis, the growing peptide chain is obtained by this method in solution. Although chemically successful, these polymers suffer several drawbacks that are mainly mechanical in nature.[7] Polar hydrophilic acrylamide[8,9] and polyvinylpyrrolidine[10]-based copolymers were introduced as an attractive alternative to the most commonly used DVB–PS to increase the hydrophilic nature of the support. Ultrahigh-loading poly[*N*-{2(4-hydroxyphenyl)ethyl}acrylamide] resin was developed in Epton's laboratory, which is suitable

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for peptide synthesis in polar solvents.[11] Small and Sherrington introduced a new composite support based on polydimethylacrylamide grafted onto highly crosslinked polystyrene for continuous flow peptide synthesis.[12] Polyethylene glycol-*graft*-PS was used as an effective polymer support in SPPS.[13,14] GMA (glycidyl methacrylate)-EDMA (ethylenedimethacrylate)[15] and crosslinked phenylacrylate[16] were introduced as improved supports with possible application in peptide synthesis. More recently, polyethylene glycol dimethacrylamide copolymers[17] and amphiphilic copoly(styrene-acrylamide)[18] were reported as hydrophilic supports for peptide synthesis.

Solid or gel phase peptide synthesis involves a heterogeneous reaction between solvated resin-bound peptide chains and soluble activated amino acid derivatives in proper solvents.[19] It is of interest for polymer chemists, particularly peptide chemists, to understand the physical and chemical properties of these components to achieve the goal of the ideal peptide synthetic condition. The effect of the macromolecular character and extent of crosslinking on the reactivity of the attached amino group in differently crosslinked polyacrylamide (PA) support was investigated by following the aminolysis reaction of *p*-nitrophenyl acetate by differently crosslinked amino PAs.[20] Acrylamides crosslinked with *N*,*N*-methylenebisacrylamide (NNMBA) tetraethylene glycol diacrylate (TTEGDA), and DVB in different proportions were employed. It was shown that the amino polymer derived from TTEGDA-PA copolymer showed a significant increase in reactivity of the amino group followed by NNMBA-PA and least by DVB-PA support. These studies clearly show the necessity for the judicious selection of structural variables for the design of tailor-made polymer for use as supports for peptide synthesis.

Pickup et al. made direct measurements of the self-diffusion coefficient of the coupling of symmetric *tert*-butoxycarbonyl(Boc)-amino acid anhydride in CH_2Cl_2 with 1% crosslinked in functionalized PS beads in CH_2Cl_2 by the NMR pulsed-gradient spin-echo method.[21] These studies showed that mass transfer did not appear to be a limiting factor because the rate of reaction did not depend on the speed of stirring of the slurry of PS beads in the solution. The studies on the gel phase reaction kinetics revealed the fact that not all reaction sites in the swollen beads are equally reactive or equally accessible to external reagents. The reaction sites for the first 90% reaction are effectively homogeneously distributed throughout the bead, but there could be substantial deviation to a slower rate during the final 10% reaction. DVB crosslinking of a polymer can cause the final stage of reaction to be extremely slow and sluggish. An effective polymeric support for repetitive synthesis of biopolymers, particularly peptides, should facilitate the different types of organic reactions occurring in both polar and nonpolar mediums. This is possible only in the case of a macromolecular matrix with optimum hydrophobic–hydrophilic balance. In addition, the crosslinked polymer matrix should be mechanically stable to withstand the multitude of synthetic operations. The DVB-crosslinked PS has the necessary mechanical stability, whereas the PA system lacks this. To provide the above essential characteristics of an effective polymeric support in one matrix, the feasibility of developing a crosslinked polymer support consisting of a hydrophobic PS chain and flexible hydrophilic crosslinking agents was considered.[22] This article discusses the preparation of such a type of polymer support that contains PS backbone and TTEGDA crosslinks, its functionalization, characterization, swelling, reactivity studies, and illustration of the application of this resin in peptide synthesis.

EXPERIMENTAL

Materials

Dicyclohexylarbodiimide (DCC) and Boc-carbazate were purchased from Sigma Chemical Company (St. Louis, MO). Styrene and TTEGDA (Aldrich) were distilled under vacuum before use. Boc amino acids were prepared according to a reported procedure.[23] Chloromethylmethyl ether was prepared[21] and all solvents were purified according to literature procedures. Polyvinyl alcohol (PVA; MW 72,000; 88% hydrolyzed) was used as received.

Instruments

IR spectra were recorded on a Shimadzu IR 470 Spectrophotometer in KBr pellets. The ^{13}C-cross-polarization-magic angle spinning (CP-MAS) solid-state NMR measurements were conducted on a Bruker 300 MSL CP-MAS instrument, operating at 75.47 MHz. The spectra were run with a fine powder of polymer beads at room temperature. The samples were spun with a Kel-F rotor at 3.35–3.39 kHz and the spectral width employed was 25 kHz. The cross-polarization time was 1 ms, and the number of scans was within the range of 200–1000. Each sample was rotated with two different spin routes; and by com-

paring the resultant spectra, the spinning side bands were eliminated. ^{1}H-NMR spectra were recorded on a Bruker WL 270 NMR instrument. Scanning electron micrographs (SEMs) of the copolymer beads were taken on a StereoScan 250 WAX 2A Cambridge instrument. The amino acid analysis was carried out on an LKB 4151 Alpha plus amino acid analyzer. For this analysis the peptide was hydrolyzed using 6*N* HCl-TFA (2 : 1 ratio) in a Pyrex glass tube under nitrogen for 15 h at 130°C.

Suspension Polymerization

In a typical experiment a four-necked reaction vessel equipped with a thermostat, Teflon stirrer, water condenser, and nitrogen inlet, and a dropping funnel were used. PVA (2.7 g) dissolved in double distilled water (300 mL), calcium sulfate (50 mg), and calcium phosphate (50 mg) were added to the vessel. A mixture of styrene (33.32 g) TTEGDA (4.02 g) and benzoyl peroxide (0.5 g) dissolved in toluene (50 mL) was added to the vessel by stirring the aqueous solution at 400 rpm. A slow stream of nitrogen was bubbled into the reaction mixture. The reaction mixture was maintained at 80°C using a thermostated water bath and the reaction was allowed to continue for 10 h. After that the solvent embedded copolymer beads were washed free of stabilizer and unreacted monomers by treating with distilled water, acetone, chloroform, and methanol. The copolymer was further purified for 6 h by refluxing with trifluoroacetic acid (TFA) to remove any linear polymer. The polymer beads were filtered, washed with CH_2Cl_2 (6 × 150 mL), CH_3OH (6 × 150 mL), and ether (6 × 150 mL) and dried under vacuum at 50°C for 10 h to yield dry beads (31.7 g, 85%) in a size range of 80–150 μm.

IR (KBr): 1720, 1490 cm^{-1} (ester); 1150 cm^{-1} (ether); 690 and 755 cm^{-1} (aromatic).

Chloromethylation

A mixture of TTEGDA-crosslinked PS resin (5 g), chloromethyl methyl ether (30 mL), and CH_2Cl_2 (25 mL) was stirred for 5 min followed by addition of 1.0*M* $ZnCl_2$ in THF (2 mL). The suspension was refluxed at 45–50°C for 10 h. The resin was filtered and washed with CH_2Cl_2 and THF/H_2O, THF/3*N* HCl, and THF and CH_3OH. Drying under vacuum at 40°C for 10 h yielded 5.6 g of chloromethyl resin. The resin was found to have a capacity of 2.5 mmol Cl/g as estimated by Volhard's method.[25]

IR (KBr): 1720 and 1480 cm^{-1} (ester); 1258 cm^{-1} CH_2Cl; 1150 cm^{-1} (ether).

Swelling

Solvent imbibition of the various resins was determined by a centrifuge method. A sample of the resin (1 g) was placed in a glass-sintered stick, porosity No. 3, and the latter immersed in the solvent for 1 h. The stick was then transferred to a centrifuge tube where it was held in position. Excess solvent was removed by centrifuging for 15 min. The stick and the contents were then weighed. Immersion in the solvent was continued for 5 min. These operations were repeated until a constant weight increase was achieved. Finally, a similar blank experiment was performed using an empty sintered stick. The data was expressed as the volume of solvent absorbed by unit weight of dry resin (mL/g). In addition, the volume occupied by unit weight of dry resin in its solvent swollen state (mL/g) was measured by noting the volume resulting when a definite weight of dry resin was added to a known volume of solvent in a small measuring cylinder.

Stability Studies

Resin samples, 500 mg of each, were separately stirred for 36 h with the following reagents: 4*N* HCl-dioxane (10 mL); 10% diisopropyl ethylamine (DIEA)/triethylamine(TEA) in CH_2Cl_2 (10 mL); 50% TFA in CH_2Cl_2 (10 mL), and 50% piperidine in DMF (10 mL). The resin samples were filtered, washed thoroughly with ethanol, water, acetone, CH_2Cl_2, dioxane, and ether; dried, and weighed (500 mg).

IR (KBr): 1720, 1480 cm^{-1} (ester); 1150 cm^{-1} (ether); 690 and 755 cm^{-1} (aromatic).

Reactivity Studies

p-Nitrophenyl acetate (50 mg) was dissolved in dioxane (5 mL) and diluted to 100 mL with water; 1 mL of this solution was withdrawn, made up to 10 mL, and used as the blank solution. The amino resin (0.5 mmol) was added to this solution and shaken well. After 5 min optical density (OD) was measured spectrophotometrically (400 nm). The experiment was repeated at different intervals. The standard curve was constructed plotting the OD against concentration. From the curve, the amount

of *p*-nitrophenol liberated at each time interval was determined. The rate constant for the reaction was calculated using the second-order rate equation.

Peptide Synthesis

Cesium Boc Alanate

Boc-Ala (0.945 g, 5 mmol) was dissolved in 4 : 1 ethanol/water (7 mL) and a 1 *M* solution of Cs_2CO_3 was added dropwise until the pH was 7.0. The solvent was removed by azeotropic distillation with benzene and the resulting white solid was kept overnight over P_2O_5 under vacuum.

Boc-Ala Resin

Cesium *t*-butyloxycarbonyl alanate from the above step was dissolved in dry DMF (7 mL) and chloromethyl resin (1 g, 2.5 mmol Cl) swelled in DMF was added to this. The mixture was kept at 40°C for 15 h with occasional shaking. The resin was washed with DMF (3 × 1 min), DMF/water (9 : 1) (5 × 2 min), DMF/water (4 : 6) (5 × 2 min), DMF (3 × 1 min), CH_2Cl_2 (3 × 2 min), and finally with ether. The product resin was dried under vacuum for 8 h (1.5 g). Amino acid analysis of the resin hydrolyzate indicated a capacity of 1.8 mmol of Ala/g resin.

A resin with 1.8 mmol of Boc-Ala/g was used for the synthesis. Boc-Leu and Boc-Ala were coupled by the DCC procedure. Each coupling was performed twice. The couplings were monitored by Kaiser's semiquantitative ninhydrin test. Solid phase reactions were carried out on a mechanical shaker in a glass vessel equipped with a fretted disk and a stopcock. One cycle of synthesis based on 0.46 g of starting resin consisted of the following operations: CH_2Cl_2 wash, 10 mL, 3 × 1 min; deprotection: 30% TFA in CH_2Cl_2, 10 mL, 30 min; CH_2Cl_2 wash, 10 mL, 3 × 2 min; DMF wash, 10 mL, 3 × 2 min; prewash 5% DIEA/DMF, 10 mL, 1 × 2 min; neutralization: 5% DIEA/DMF 10 mL, 1 × 10 min; DMF wash, 10 mL, 3 × 2 min; CH_2Cl_2 wash 10 mL, 3 × 2 min; equilibration with Boc-amino acid (2.5 mmol), 7 mL CH_2Cl_2, 10 min; DCC in 5 mL CH_2Cl_2 (2.5 mmol), 45 min; 30% ethanol/CH_2Cl_2 wash, 10 mL, 3 × 2 min; and CH_2Cl_2 wash, 10 mL, 3 × 2 min. At the end of the synthesis, the resin was washed with methanol and dried and 1.5 g of dry peptide resin was obtained. About 60 mg of the resin was used for the ninhydrin test during the course of the synthesis.

Cleavage of Boc(Ala-Leu-Ala)4-OMe from Solid Support by Transesterification

A suspension of the peptide resin (0.7 g) in 30 mL of anhydrous methanol and 4 mL of TEA was stirred under reflux for 4 h. The resin was filtered and the methanolic solution evaporated to yield the crude peptide. Two cycles of transesterification were carried out to ensure complete recovery of the peptide. 360 mg crude peptide was obtained from 700 mg of the peptide resin. The crude peptide was partially soluble in warm DMSO. The peptide was purified by reprecipitation from DMSO–diethylether mixture. A single spot was obtained on thin-layer chromatography (TLC).

Rf(A): 0.76; Rf(B): 0.66.

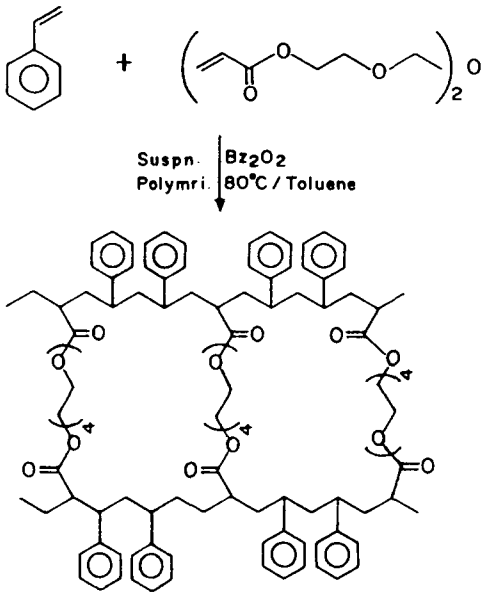
The solvent system used for TLC: A 1-butanol : acetic acid : water : ethyl acetate (1 : 1 : 1 : 1); B: 1-butanol : acetic acid : water (6 : 1 : 5).

ANAL (AMINO ACID): Ala, 2.05 (2); Leu, 0.98 (1); NMR (DMSO-d_6, 270 MHz), δ 1.44 (*s*, 9H, Boc); 0.95 (*t*, 24H, CδH Leu); 1.37–1.52 (m, CγH and Cβ, Leu); 1.25 (*d*, 24H, CβH Ala); 3.6 (3H, OCH_3); 4.13–4.35 (8H, CαH, Ala, 4H, CαH Leu); 7.5–7.92 (8H, —C—NH Leu-Ala), 6.65 (1H, Boc NH Ala).

RESULTS AND DISCUSSION

The chemical nature and topographical structure of the polymer matrix are the two important factors that determine the physicochemical properties that render a polymer support favorable for peptide synthesis. The main aim of the current study was the search for monomers that give a polymer that interacts with a broad spectrum of solvents and development of a reproducible procedure for obtaining beaded resins. The topography of the polymer matrix is determined by the chemical nature of the monomers and the mole percentage of crosslinking agent. The crosslinking provides the desired mechanical integrity for the resins.

Suspension polymerization was proved to be the most useful technique for synthesizing crosslinked polymeric supports, principally because of the extremely convenient physical form of the beaded product that lends itself to further conversion.[26,27] PS samples with 4 mol % TTEGDA were prepared by aqueous suspension of monomers and toluene as diluent at 80°C using benzoyl peroxide as initiator (Scheme 1). High molecular weight PVA or



Scheme 1. Suspension polymerization of styrene and TTEGDA.

poly(vinyl pyrrolidone) was used as the suspension stabilizer. Beads of convenient shape and size could be obtained under these conditions. The polymer beads were obtained in high yield and particle size ranging from 80 to 150 μm. They showed good mechanical properties and were obtained in easily filterable form. Reproducible results could be obtained in the case of particle size distribution of the polymer beads by adjusting the amount of suspending agents, geometry of vessel, stirring rate, and shape of stirrer. The polymer beads were refluxed with TFA to remove any linear polymeric impurity and monomers. An SEM of the typical copolymer beads is shown in Figure 1. The micrograph shows that all particles are perfectly spherical and almost uniform in their size. The shapes of the particles were inferred from the SEM patterns. Guyot et al. extensively used the SEM technique for studying the morphological features and mechanism of formation of the beads.[28,29]

Functionalization/Characterization of Polymeric Support

Functionalization of polymers involves polymerization of substituted monomers directly or functionalizing the preformed polymer. Introduction of functional groups into PS by copolymerization of suitably substituted styrene monomers gives polymers of more uniform functionalization. In addition, they are not contaminated by small proportions of other functional groups remaining from incomplete prior chemical transformation.[30] However, in the case of functionalization of the preformed polymer, the functional groups will be more accessible to reactants for further chemical modification.

TTEGDA-crosslinked PS was functionalized by electrophilic substitution of the aromatic ring. Chloromethylation of the styrene ring was carried out using chloromethyl methylether in the presence of Lewis acid and CH_2Cl_2 as the solvent. Chloromethylmethyl ether can be conveniently prepared in good purity by passing dry HCl gas through a methanol-formaldehyde mixture.[24] Anhydrous $SnCl_4$ is found to be a good catalyst in chloromethylation reactions at low temperature. In the present case, anhydrous $ZnCl_2$/THF was used as the catalyst for the controlled chloromethylation reaction.[31] The reaction can be easily controlled and chloromethyl PS of the desired chlorine capacity can be prepared by varying the amount of reagent, catalyst, temperature, and duration of reaction. The results are given in Table I.

Adequate characterization of the polymeric support, and the functional group conversion carried out on the support, is a major problem associated with polymer-supported chemistry. Crosslinked macromolecular supports are highly insoluble and methods such as UV and NMR present major problems for providing detailed structural information. IR spectroscopy is the most widely used technique, not only in following polymer supported reaction, but also for structural identification.[32] TTEGDA-crosslinked PS was characterized by IR spectroscopy. IR spectra were recorded in solid KBr pellets because all polymers were insoluble. TTEGDA-crosslinked PS resin shows an intense peak at the 1720 cm^{-1} band of the ester carbonyl and a band at

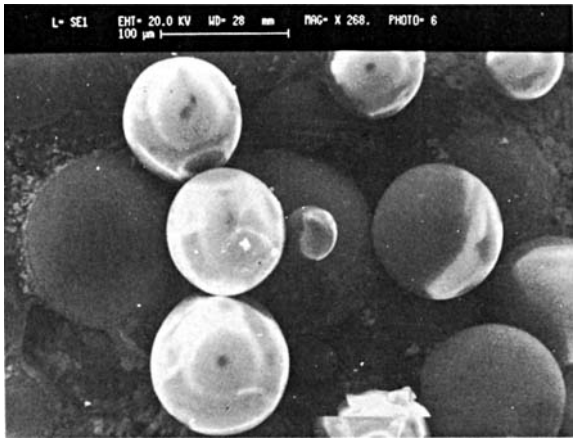


Figure 1 Scanning electron micrograph of TTEGDA-PS resin.

Table I Chloromethylation of TTEGDA-Crosslinked PS Using $ZnCl_2$/THF Catalyst

Duration of Reaction (h)	Temperature (°C)	CH_2Cl Substitution (mmol/g Resin)
1	50	0.25
2	50	0.51
3	50	1.04
4	50	1.25
5	50	2.05
1	40	0.10
2	40	0.18
3	40	0.54

Catalyst was 0.5 g resin, 0.1 mL 1.0*M* $ZnCl_2$ in THF, 3 mL $ClCH_2OCH_3$, and 2.9 mL CH_2Cl_2.

1150 cm^{-1} of the ether linkages of the crosslinking agent besides the usual peaks of PS [Fig. 2(a)]. These peaks were retained under all conditions of SPPS. In chloromethyl TTEGDA–PS resin, there is a sharp band at 1258 cm^{-1} corresponding to H—C—Cl bonding vibration [Fig. 2(b)].

Gel phase ^{13}C-NMR was employed to monitor SPPS on most commonly used PS-based resins.[33] This technique proved to be useful for characterizing PS-based starting supports as well as for determining the degree of functionality and purity. DVB-crosslinked phenyl acrylates were characterized by the solid state ^{13}C-CP-MAS-NMR method.[16] TTEGDA-crosslinked PS resin was further characterized by ^{13}C-CP-MAS (solid state) NMR spectroscopy. The solid-state ^{13}C-CP-MAS-NMR spectrum of TTEGDA-crosslinked PS showed an intense peak at 127.9 ppm, which corresponds to aromatic PS rings, and a small peak at 145.6 ppm, arising from the C-3 carbon of the PS ring. The backbone methylene carbon of the polymer appears as a single peak at 40.3 ppm. The methylene carbon of the ether linkage of the crosslinking agent, TTEGDA, appears as a small peak at 70.6 ppm [Fig. 3(a)]. Chloromethyl resin was also characterized by ^{13}C-CP-MAS-NMR method. Chloromethyl resin gave an additional peak at 46.1 due to the methylene carbon atom of the chloromethyl group and a small peak appears in the region 135.6 ppm, corresponding to the C-6 carbon of the PS ring [Fig. 3(b)].

Swelling and Stability Studies of Polymer Support

For the success of SPPS, the accessibility of the growing resin-bound peptide chain to reagent and solvents is very important. For maximum accessibility of the reactive functional group in the resin, the polymer matrix should swell extensively in the solvating medium.[34] This measure of swelling property of a polymer support is a criterion for its efficiency in solid phase synthesis. There are two main classes of supports, the gel type resin and macroporous resin. The gel type resins are generally lightly crosslinked (1–5%) and appear translucent. They have no permanent porosity but swell in various organic solvents. The space between the crosslinks occupied by the solvent are considered as small pores in these resins. Macroporous resins are obtained when the polymerization is carried out with higher (>5%) amounts of crosslinking agent in the presence of porogens. In the present study, microporous TTEGDA–PS and DVB–PS resins were prepared by suspension polymerization under identical conditions and swelling studies were carried out. Four percent TTEGDA-PS resin showed effective swelling in polar as well as nonpolar solvents when compared to the 1% DVB–PS resin. To investigate the effect of the hydrophilic crosslinking agent (TTEGDA), the swelling characteristics of the resin in solvents of varying polarity were measured. The swollen volumes of the resin in different solvents are given in Table II. Chloromethyl TTEGDA-crosslinked PS also shows the same extent of swelling as the original TTEGDA-crosslinked PS resin, confirming that there was no additional crosslinking

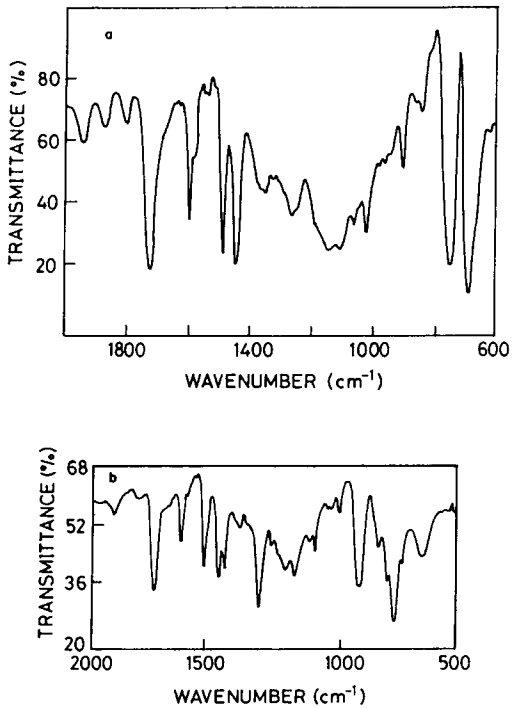


Figure 2 IR spectra of (a) TTEGDA–PS resin and (b) chloromethyl TTEGDA–PS resin.

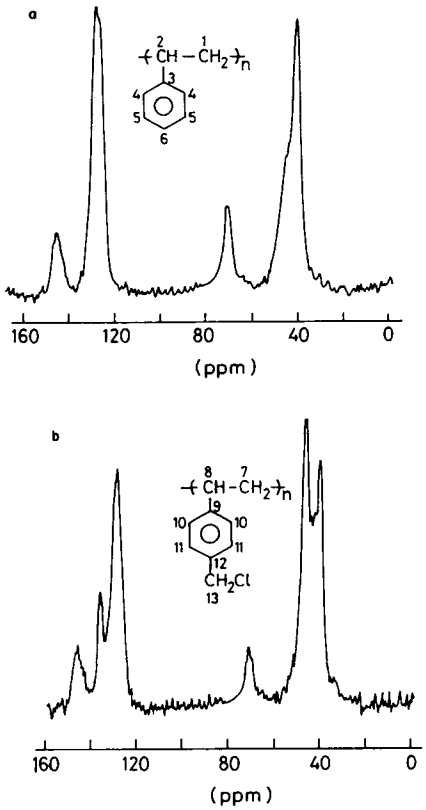


Figure 3 ^{13}C-CP-MAS-NMR spectra of (a) TTEGDA-PS resin and (b) chloromethyl TTEGDA–PS resin.

during chloromethylation. The volume occupied by the unit weight of the dry chloromethyl resin in different solvents are 5.8 mL (DMF), 8.8 mL (CH_2Cl_2), 9.1 mL (*N*-methyl-2-pyrrolidone, NMP), 7.2 mL benzene, 8.7 mL dioxane, and 8.7 mL THF. Swelling measurements were carried out by placing the beads in a graduated cylinder with excess solvent and noting the initial and final volume of the beads. The results show that the TTEGDA-crosslinked PS resin has enhanced swelling behavior in polar solvents. This helps maximum diffusion of soluble reactants and reagents into the polymer matrix and facilitates the reaction.

Another important criterion for a solid support to be suitable for peptide synthesis is that the resin should be mechanically stable under all conditions of repeated synthetic operation and the parts of the support other than the functional group should be chemically inert during the various synthetic reactions. The 4% TTEGDA-crosslinked PS resin was found to be stable even after vigorous conditions of functionalization. The new polymer support has comparable physical and mechanical properties as that of DVB-crosslinked PS support, permitting identical manipulation such as shaking and filtration when used as a support for SPPS. To test the stability of the resin under various conditions of peptide synthesis, the resin was subjected to the commonly encountered conditions in peptide synthesis. The resin should have enough stability to withstand the repeated acid treatment required for the Boc removal and base treatment of 9-fluorenylmethyloxycarbonyl(Fmoc) removal. The stability of the resin was tested under the acidic and basic conditions of Boc removal and Fmoc removal. There was no degradation of the polymer under these conditions and an IR spectrum of the resin after treatment showed no peak due to the carboxylic acid group, showing that no hydrolysis of the ester crosslinks had occurred. These observations indicate that the crosslinks are stable enough for repeated synthetic steps. Similar observations were also made in the case of resins obtained from GMA crosslinked with ethylene glycol dimethacrylate.[35]

Reactivity Studies

To study the reactivity of the attached functional group, aminolysis of *p*-nitrophenyl acetate with amino methyl resin was taken as the model reaction. Amino methyl PS resins were prepared according to a literature procedure.[20] The aminolytic reaction was followed spectrophotometrically. Since the polymer formed a separate phase in the present case, it was difficult to calculate the actual rate constant. The method of log linear least squares was used to fit the data to second-order kinetics. In all the 6 cases, the slope m and hence the rate constant k were calculated. The correlation coefficients (γ) were also calculated. The results are given in Table III. The values approaching unity for γ suggest that

Table II Comparison of Swelling Characteristics of Crosslinked Polystyrene Gels

Solvent	Swelling Factor[a] 1% DVB-PS	4% TTEGDA-PS Resin (mL)
Chloroform	4.3	6.5
Tetrahydrofuran	5.2	8.7
Toluene	4.7	6.9
Pyridine	4.2	6.0
Dioxane	3.5	8.8
Dichloromethane	4.3	8.8
DMF	2.8	5.5
Methanol	1.8	2.2

[a] Determined by gain in weight of solvent/g dry beads reduced to volume of solvent/g dry beads.

Table III Kinetics of Aminolysis of *p*-Nitrophenyl Acetate with Aminomethyl PS

Resin	Crosslink Density (mol %)	Rate Constant (L mol^{-1} min^{-1})	Correlation Coefficient, γ	% Aminolysis (After 30 min)
PS/DVB	1	1.86	0.993	47.9
	2	1.51	0.992	40.2
	3	0.75	0.979	26.2
	4	0.09	0.992	6.5
PS/TTEGDA	4	2.50	0.985	65.9

the experimental rate constant agrees with second-order kinetics. The rate constant for aminomethyl PS with 4% TTEGDA was 2.50 L mol^{-1} min^{-1}, whereas that for aminomethyl resin crosslinked with 4% DVB was 0.75 L mol^{-1} min^{-1}. The extents of reaction after 30 min were 65.9 and 26.2%, respectively. The increased reactivity arises from the increased flexibility of the styrene-TTEGDA copolymer. The soluble species can easily penetrate into the polymer matrix, thereby increasing the rate of reaction. The compatibility of the substrate and the solvent appears to be the most important factor responsible for this. In highly crosslinked styrene–DVB polymers, the rigidity of the polymer backbone also contributes to the reduced reaction rate.

Peptide Synthesis

Authoritative working recommendations in the literature tell us that SPPS is best carried out using low initial matrix loading, typically 0.1–0.3 mmol amino acid/g resin.[2] Strict adherence to these recommendations has discouraged experimentation in situations in which higher initial matrix loading would have been quite practicable. Sarin et al. showed that for the PS-DVB based SPPS of a large polypeptide, the swollen volume matrix–peptide assembly in common peptide solvents increased dramatically with increasing peptide content and that breakdown in synthetic capacity did not occur.[36] Although it may be imprudent to infer that similar increases in swelling volume occur during SPPS of all peptides, these observations have an important corollary. It is implicit that, for most peptide targets, it should be possible to select the crosslink density/chemical nature of crosslink of the matrix so that there is ample volume to accommodate the target peptide.

In the case of PS–TTEGDA resin, because of the highly flexible and long crosslinks, it is possible that there will be enough available volume for the growing peptide chain even at high capacity; hence it was thought of synthesizing the hydrophobic 12-residue peptide using high capacity chloromethyl TTEGDA-crosslinked PS solid support. Boc-Ala resin (1.8 mmol Ala/g) was prepared by the cesium salt procedure in high capacity from chloromethyl resin.[37] This resin was taken in a solid phase reaction vessel and the remaining amino acid residues were incorporated in the manual mode. *N*-Boc protection was used throughout the synthesis. Boc amino acids were coupled by the DCC method. After each coupling reaction, the dicyclohexylurea formed was removed by washing with 30% EtOH–CH_2Cl_2 mixture. Completion of the coupling was confirmed by the semiquantitative ninhydrin method.[38] Removal of the Boc group was effected by 30% TFA in CH_2Cl_2. The solvation, swelling, and the overall size of the polymer beads increased with the elongation of the peptide chain. There was a 2.5-fold weight increase in the peptide resin after the synthesis. This agrees approximately with the molecular weight of the target peptide. The SEM showed an increase in bead size after the synthesis. This increase in the size of the beads is due to the accommodation of the peptide chain in the high capacity TTEGDA-crosslinked PS resin. The high capacity resin has the advantage of obtaining peptides in relatively large amounts as in the solution phase method.[39] Monitoring of the reaction by the ninhydrin test is much easier in these high capacity and highly swelling resins. In the ^{13}C-CP-MAS-NMR spectra (Fig. 4) of the peptide resin, the intensity of the peaks in the methylene region of the peptide chain is enhanced and the styrene carbon peaks are diminished, showing that the peptide is the major component in the polymer matrix.

The cleavage of the peptide from the solid support was accomplished in high yield (95%) by transesterification with anhydrous methanol–TEA at 50°C. Although cleavage by anhydrous hydrofluoric acid has been extensively used in SPPS, considerable difficulties were experienced in the purification of hydrophobic peptides in these cases. Apart from generating protected peptides suitable for postsynthetic modification for physicochemical studies, the transesterification method of cleavage also aids in pu-

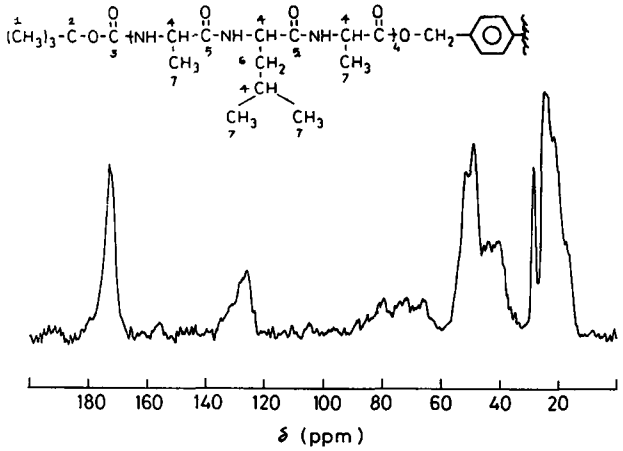


Figure 4 75.47 MHz ^{13}C-CP-MAS (solid-state) NMR spectrum of Boc(Ala-Leu-Ala)$_4$ TTEGDA–PS resin. LB, 6 Hz; NS, 1035; δ (ppm)-assigned carbon: 172.6 C-5, 156.2 C-3; 126.0 C-styrene ring, 79.2 C-2; 71.4 C-ether linkages of polymer; 55.2–48.7 C-4/6; 40.2 C-polymer backbone methylene; 28.4 C-1; 15.6–25.4 C-7.

rification because the protected peptide esters are relatively more soluble in organic solvents.[40] But in the present case the peptide was highly insoluble due to the extreme hydrophobic nature of the peptide. Hence purification could not be carried out by the usual chromatographic techniques. The crude product was partially soluble in warm DMSO. Hence purification was carried out by reprecipitation in the DMSO/ether mixture and washing off the soluble impurities. The final product shows a single spot on TLC and was identified as the target peptide by amino acid analysis and by ^{1}H-NMR (Fig. 5).

CONCLUSION

A new resin was developed for peptide synthesis by a systematic analysis of solvation, swelling, and reactivity characteristics of functionalized crosslinked polymeric supports considering the existing problems in SPPS. This resin, which could be conveniently prepared by suspension polymerization of styrene and TTEGDA, serves as a new class of polymer support for peptide synthesis. These supports are extremely stable under all conditions of peptide synthesis and they have added advantages of high swelling and increased reactivity in the aminoacylation and deprotection steps driving the gel phase reaction to near completion. The advantage of PS–TTEGDA resin over ultrahigh load polymer[11] lies in its ease of preparation, functionalization, and workup procedure. The main advantages of this support are compatibility with the growing peptide chain and optimum hydrophilic–hydrophobic balance. Compared to the classical SPPS at conventional loading,[2,9] these high capacity resins have a number of advantages, which stem from much more efficient use of available volume within each gel particle. These advantages include enhanced coupling rate during peptide bond formation, major saving in cost due to more effective use of reagents and reaction and washing solvents, greatly improved sensitivity in the monitoring of coupling reaction to effect peptide bond formation, and the synthesis of peptides in quantities approaching those obtained by the solution phase method.

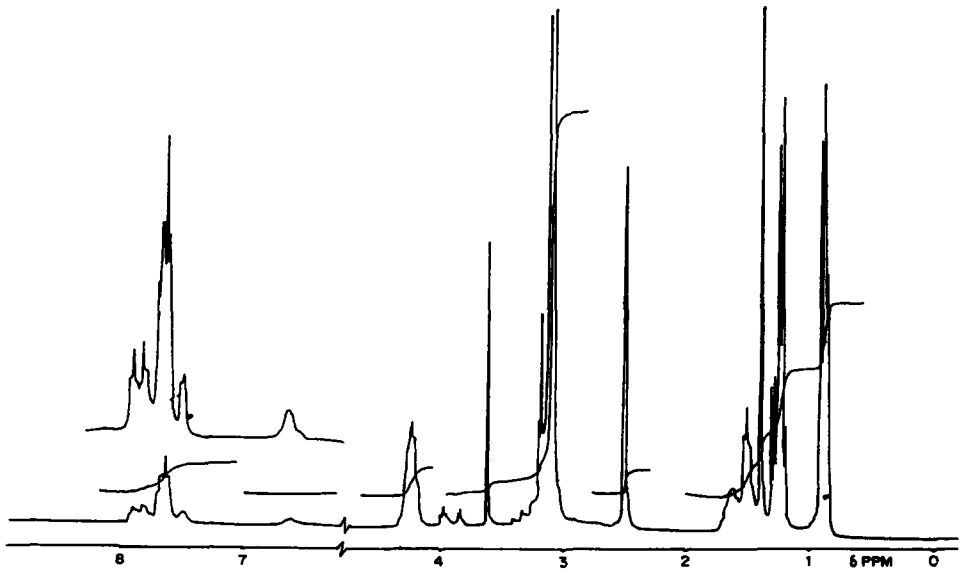


Figure 5 ^{1}H-NMR spectrum of Boc(Ala-Leu-Ala)$_4$-OMe.

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