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Perspective Article

Multifunctional polyether grafted dendritic solid supports: Novel class of solvent like high loading hybrid supports for solid phase organic synthesis

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ABSTRACT

Three novel multifunctional polyether grafted dendritic solid supports for organic synthesis were developed via single step, grafting from and hypergrafting strategies. In order to improve both the swelling capacity and loading capacity of conventional resins simultaneously, the hybrid supports were developed by grafting either multivalent linear polymers or dendritic architectures to the conventional resin. The ring opening polymerization of strained cyclic monomers was explored for the development of multivalent polymer grafts on the solid support. The physico-chemical properties of the dendritic solid supports were studied by various characterization techniques and analytical methods. Among the hybrid supports, Het-PG was found to be a novel solvent like high loading solid support. High functional group capacity of 7 mmol g^{-1} which was introduced via single step approach, increased accessibility of functional sites even at high loading etc. make Het-PG a versatile solid support for organic synthesis. The applicability of dendritic solid support (Het-PG) as a high loading hybrid support for multistep solid phase organic synthesis of small peptides was investigated. The high functional group capacity and increased accessibility of functional sites in Het-PG were exploited for addressing the poor industrial scalability issue of solid phase organic synthesis. A novel approach to solid phase peptide synthesis using solvent like high loading hybrid solid support is presented.

1. Introduction

In recent years, instead of conventional crosslinked resins, dendritic solid supports have gained considerable attention due to their improved properties such as high functional group capacity, increased accessibility of functional sites in the reaction medium etc [1–3]. Grafting long polyethylene glycol chains to resin support is a well established method for improving swelling capacity of hydrophobic resins. Due to the amphiphilic nature of PEG chains, PEG grafted supports, especially, the most accepted crosslinked polystyrene based resins, exhibit very high swelling capacity in both polar and nonpolar solvents [4–7]. The flexible PEG grafted supports have been reported to provide a more or less solution-like environment to the bound substrates [8]. But as a result of PEG grafting, functional group capacity of the resin was reduced drastically. This is because of the fact that, during PEG grafting, mass of the resin increased considerably, while the number of chemically reactive sites within the resin remained constant.

Grafting multivalent linear polymers or dendritic architectures to the resin is a better solution to overcome the above mentioned difficulties. As a reflection of this concept, dendronized solid supports have been introduced as high loading supports for organic synthesis [9]. Although the functional group capacity per gram was not increased much, the loading per single bead showed dramatic increase during the dendronization process [10]. Also, the high density and polarity of functional groups within the resin bead generated after grafting dictated the swelling behaviour of the support [2,3]. With the aim of improving the swelling properties and loading capacity of solid supports, many dendronized solid supports with varying dendron structure and generation were developed during the past few years [11–23]. A typical PAMAM-type dendronized polystyrene resin developed by Fromont et al. showed a superior loading of 230 nmol per bead and swelling capacity of 9 mL g^{-1} at second generation level itself [24]. But the tedious multistep synthesis limits the practical applicability of dendronized solid supports in organic synthesis.

The dendritic solid supports developed via single step grafting from approach or hypergrafting method have received special attention in modern periods. Recently, Zhang et al. reported a branched, poly (ethylene glycol) acrylate grafted polystyrene resin (PS-PEGA) as high

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loading support for organic synthesis [25]. The support was developed by grafting poly (ethylene glycol) acrylate monomer from Merrifield resin via single step activators generated by electron transfer atom transfer radical polymerization (AGET- ATRP). The resulting hybrid support exhibited good swelling property in both polar and nonpolar solvents. The swelling capacity of grafted support in polar solvent was found to be two times higher than that of Merrifield resin. The functional group capacity of the hybrid support was reported to be 0.5–1.2 mmol g^{-1} which was almost five times higher than that of conventional PEG grafted polystyrene resin (PS-PEG). The solid phase multistep organic synthesis demonstrated using the hybrid support clearly suggested the good accessibility of functional sites in the reaction medium.

The ring-opening metathesis polymerization of norbornene derivatives initiated on a polystyrene support was reported as an efficient method for the synthesis of high loading hybrid support for organic synthesis [26]. Eventhough the swelling characteristics were not as good as those of conventional resins, the resulting hybrid support (ROMP-spheres) showed a high functional group capacity of 3.03 mmol g^{-1}. Rasta silane resins, developed by the TEMPO-methyl resin initiated living free radical polymerization of dialkylsilanestyrenes were introduced as high loading support for organic synthesis [27]. Since the linear polymer graft represented the majority of the mass of the bead, Rasta silane resin was expected to have a special structure with multivalent linear polymeric chains extending beyond the original bounds of the bead. The authors also presented the convenient multistep organic synthesis and selective cleavage of the product from the support. So in addition to high loading capacity, the high accessibility of functional sites during organic synthesis was also realized in this support.

Hypergrafting strategy is a versatile method for the introduction of large number of functional groups within one step and the method offers easy one step synthesis of dendritic solid supports [1]. The resulting dendritic solid support closely resembles dendronized solid supports in properties. Also due to grafting, swelling property of solid supports can be modified. Some dendritic solid supports have been reported to exhibit a more solution-like reactivity of the bound substrates [28]. In addition to high functional group capacity and increased accessibility of functional sites, the dendritic solid support provides a convenient platform for utilizing the special properties of dendritic architecture in organic synthesis.

Polystyrene-*graft*-polyglycerol (PS-g-PG) resin was reported by Roller et al. as the first dendritic solid support via hypergrafting strategy [28]. The hybrid support showed high functional group capacity of 4.3 mmol g^{-1} and good swelling capacity even in polar solvents. The convenient PS-g-PG resin supported multistep organic synthesis, clearly indicated the accessibility of terminal functional sites to reagents even at high loading level. This suggested a solution like environment around the substrates attached to PS-g-PG resin. Eventhough some dendritic solid supports have been introduced for heterogeneous catalysis, the applicability of these hybrid supports has not been exploited much in solid phase organic synthesis [29–37].

The main objective of the present work is the synthesis of some multifunctional polyether grafted polystyrene based high loading hybrid solid supports, which can provide an almost solution-like environment to bound substrates via grafting from and hypergrafting strategies. The multivalent aliphatic polyether type graft is specially selected due to non-coordinating nature, thermal and chemical stability etc. [38] The polyether type chains also provide better hydrophilic/hydrophobic balance to hydrophobic solid supports and contribute to the accessibility of functional sites in polar medium. This was attempted by grafting multivalent linear and dendritic polyether architecture obtained by the ring opening polymerization of strained cyclic monomers such as epoxide or oxetane derivatives, to conventional crosslinked polystyrene resin. In this approach, with the aim of improving the functional site accessibility and increasing the loading capacity of polystyrene resin simultaneously, linear polymeric chain containing functionalized ethylene glycol repeating units or the dendritic analog of polyethylene glycol/polypropylene glycol (based on carbon: oxygen ratio in the branched repeating unit) were directly attached to the resin. The applicability of dendritic solid support as high loading hybrid support for multistep solid phase peptide synthesis was investigated. An attempt has been made to extend the concept of solid phase peptide synthesis using low loading solid support to solvent like high loading dendritic solid support.

2. Materials and methods

Merrifield resin (Chloromethyl polystyrene, 2% DVB, 200–400 mesh size, chlorine capacity 1 mmol g^{-1}), bis(3-aminopropyl)amine, potassium tertiary butoxide, glycidol, 3-methyl-3-hydroxymethyl oxetane, propargyl bromide, Boc protected aminoacids, N,N′-dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt), 4-Dimethylaminopyridine (DMAP) etc. were purchased from Sigma–Aldrich. Infrared (IR) spectra of polymer samples were recorded with JASCO FTIR 4100 spectrometer using KBr pellets. Thermogravimetry (TG) was carried out using Perkin Elmer Pyris Diamond 6 thermogravimetric/differential thermal analyzer with a heating rate of 10 °C min^{-1} under nitrogen atmosphere. Energy dispersive X-ray analysis (EDAX) was performed on high-speed EDAX instrument (QUANTAX 200 with XFlash®6/30 SDD Detector) from Bruker with energy resolution <126 eV and a 30 mm^2 active window area (NCESS, Thiruvananthapuram). Solid state ^{1}H NMR and CP-MAS ^{13}C NMR spectra were recorded on Bruker 400 MHz instrument at NCL, Pune. The purity of peptide was analysed by high performance liquid chromatography (HPLC). Schimadzu HPLC instrument equipped with UV-detector and C-18 column was used for this purpose. Mass spectrum was recorded using LC-Waters e 2695 instrument with mass detector-Waters 3100.

3. Experimental procedures

3.1. Synthesis of linear polyglycidol grafted dendritic solid support PS-D-LPG

Hydroxyl terminated PS-D-OH resin beads (2 g) were suspended in dry diglyme (25 mL), taken in a 500 mL three-necked flask. After swelling for 1 h, the solution of potassium tertiary butoxide (2 g) in THF was added to deprotonate the hydroxyl groups. The mixture was stirred at 40 °C over 12 h. THF and the by product tert-butyl alcohol were distilled off. The temperature was raised to 120 °C. The hydroxyl group of glycidol was protected using ethyl vinyl ether. The resulting 1-ethoxyethylglycidyl ether (25 mL, 0.17 mol) was dissolved in diglyme (35 mL) and the solution was added drop wise to the reaction mixture. The mixture was stirred for 12 h at 120 °C and neutralized with HCl (1 M). The solid resin was filtered and the resin was washed with water, diglyme and MeOH. After soxhlet extraction with methanol, it was dried under vacuum to give yellow resin beads with an yield of 2.65 g.

3.2. Deprotection of PS-D-LPG for the synthesis of PS-D-DLPG

PS-D-LPG resin (1 g) was suspended in THF (60 mL) and stirred with dilute HCl (4 mL, 32%) for 30 min. After the reaction, the resin was filtered and washed with water and THF. The resin was dried to get a final yield of 0.91 g.

3.3. Synthesis of dendritic solid support PS-D-OXE via hypergrafting of 3-methyl-3-hydroxymethyl oxetane

To the preswollen hydroxyl terminated PS-D-OH resin beads (1 g) in DCM (50 mL), BF_3-etherate (0.87 g, 6.1 mmol) was added and stirred for 30 min. The reaction mixture was cooled and 3-methyl-3-hydroxymethyl oxetane (12.5 mL, 0.13 mol) was added dropwise with constant stirring. The mixture was stirred for 24 h at room temperature. Methanol was added to quench the reaction. The reaction mixture was

filtered and the resin was washed with saturated sodium carbonate solution, water, methanol and dichloromethane. After soxhlet extraction with methanol, the resin was dried under vacuum. Yellow resin beads were obtained with an yield of 1.35 g.

3.4. First amino acid (Boc-glycine) attachment to the dendritic solid support Het-PG

Het-PG resin (0.40 g, 2.8 mmol) was suspended in DCM: DMF (9: 1, 6 mL) taken in a 250 mL R. B. flask. After swelling for 1 h the solution of 5 equivalents of Boc-glycine (14 mmol, 2.43 g) and the same equivalents of HOBt (14 mmol, 1.88 g) in minimum amount of solvent DMF was added to the resin. After adding the same equivalents of diisopropylcarbodiimide (DIC)(14 mmol, 1.75 g), the solution of 0.1 equivalent (relative to the resin) of DMAP (0.28 mmol, 0.04 g) in minimum amount of DMF was also added and stirred at room temperature for 3 h. The resin was filtered and washed thoroughly with DMF, DCM, MeOH. After drying, yellow coloured resin was obtained with an yield of 0.46 g.

3.5. First amino acid (Boc-glycine) attachment to Het-PG-N_3 resin via click reaction

In order to prepare the propargyl ester of Boc-glycine, propargyl bromide (19.2 mmol, 2.14 mL of 80% solution in toluene) was added drop wise to the cooled reaction mixture of Boc-glycine (19.23 mmol, 3.37 g) and anhydrous K_2CO_3 (19.2 mmol, 2.66 g) in dry DMF (40 mL). After stirring for an additional 1 h at −10 °C, the reaction mixture was allowed to attain room temperature. To the reaction mixture, Het-PG-N_3 (0.40 g, 2.8 mmol), copper (I) iodide (5 mmol, 0.95 g), and DIPEA (2.5 mL) were added along with DMSO-THF (1: 1) (5 mL). The suspension was stirred at 40 °C for 24 h. The resin was collected by filtration and it was washed with, DMF, acetonitrile, pyridine, MeOH and CH_2Cl_2. After drying, yellow coloured resin was obtained with an yield of 0.90 g.

3.6. Removal of Boc group

Boc protected amino acid bound resin (1 g) was treated with 30% TFA in DCM (25 mL) for 30 min. The TFA solution was filtered and the resin was washed with DCM. The washed resin was treated with 5% DIPEA in DCM (25 mL) and 5% DIPEA in NMP: DCM mixture (1:1 *v*/v, 25 mL). The resin was filtered and washed thoroughly with DCM and dried to get free amino acid resin.

3.7. Amino acid coupling to amine terminated resin/peptidyl resin

The active ester of amino acid was prepared by adding 2.5 mmol of HOBt and 2.5 mmol of DCC to a solution of 2.5 mmol of amino acid in NMP (4 mL). The mixture was stirred for 5 min, and the DCU formed was filtered off. The filtrate was added to the amine terminated resin/peptidyl resin (1 mmol) and the mixture was shaken for 45 min. DMSO (15% of total volume of coupling medium) was added to the mixture and shaken for 15 min. At the end of 15 min, DIPEA (3.8 mmol) was added. After 5 min, the solution was filtered and the resin was washed with MeOH: DCM (33:67 *v*/v) to remove DCU and followed by DCM: NMP mixture. The resin was dried and the whole procedure was repeated to ensure complete conversion. Double coupling method was employed for each amino acid attachment. Weight of peptidyl resin based on Het-PG-N_3 was 0.60 g.

3.8. Peptide cleavage from solid support

Peptidyl resin (500 mg) taken in a R. B. flask was mixed with TFA (40 mL) and thioanisole (4 mL). The mixture was stirred for 12 h at room temperature. After 12 h the resin was filtered and washed with TFA (15 mL × 3). The filtrate and washings were collected and concentrated under reduced pressure. The peptide solution thus obtained was cooled and added drop wise with continuous stirring to ice cold ether. The precipitated peptide was washed thoroughly with ether to remove the low molecular weight organic impurities. The peptide obtained was dried. The resin was subjected to a second cycle of cleaving to ensure complete removal of peptide from the support.

4. Results and discussion

Merrifield resin assisted solid phase methodology was used for the development of all the dendritic solid supports. The bifurcated bis(3-hydroxypropyl)amine attached-Merrifield resin (PS-D-OH) was directly used for the surface initiated ring opening polymerization of strained cyclic monomers such as epoxide and oxetane derivatives. The bifurcated ligand bis(3-aminopropyl)amine was introduced to the resin in order to increase the functional group capacity and to increase the accessibility of functional groups by extending the active sites from the hydrophobic polystyrene-DVB matrix. The bifurcated bis(3-hydroxypropyl)amine attached-Merrifield resin (PS-D-OH) was developed by standard reported procedures [39]. The detailed scheme, experimental procedures and IR spectral characterization of each stage of the multistep synthesis of bis(3-hydroxypropyl)amine attached-Merrifield resin (PS-D-OH) have been given in supporting information (SI-Scheme 1, and SI-Fig. 1).

4.1. Synthesis of linear polyglycidol grafted dendritic solid support PS-D-LPG

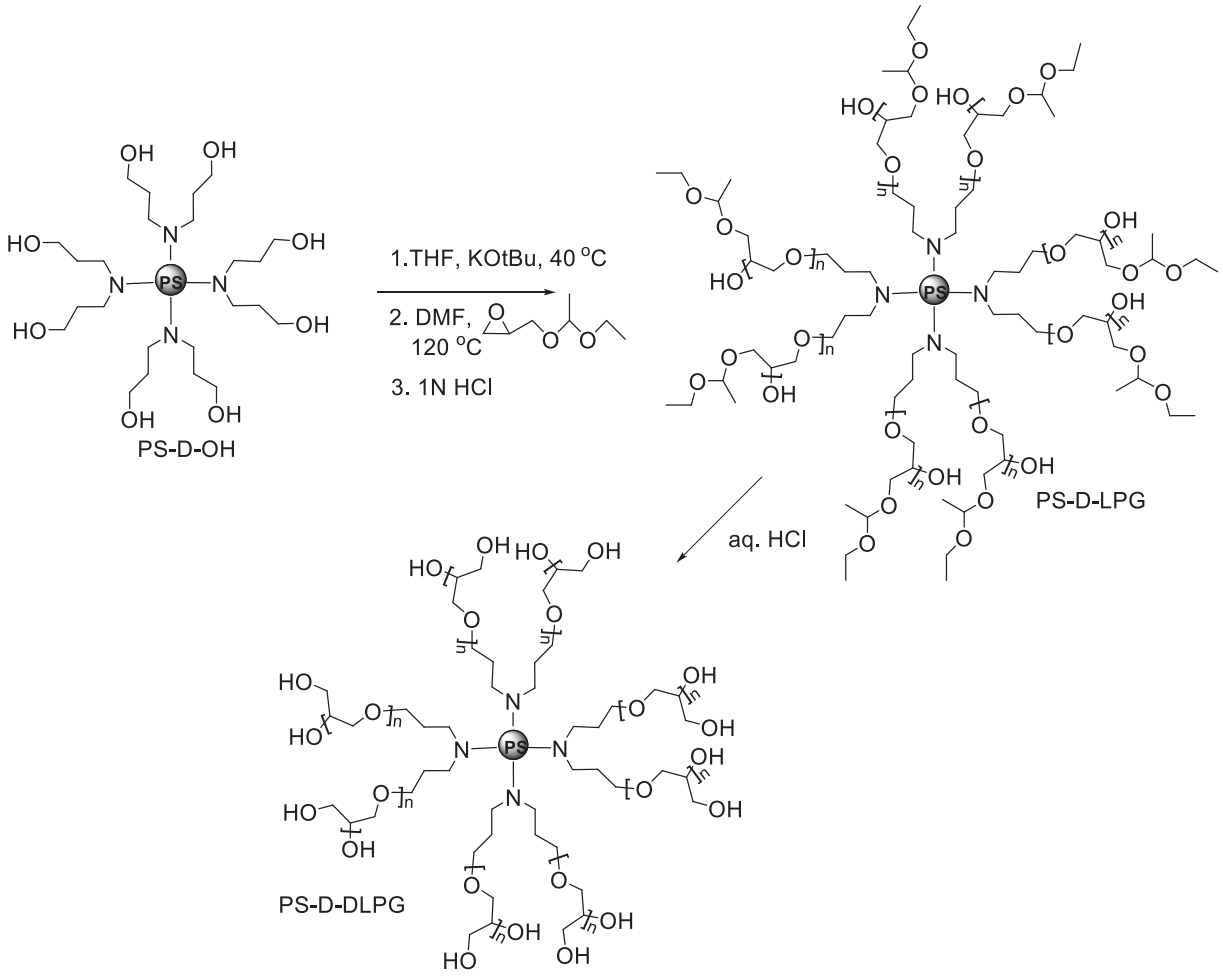
In order to get linear polyglycidol grafted dendritic solid support with high grafting efficiency, the bifurcated bis(3-hydroxypropyl)amine attached-Merrifield resin (PS-D-OH) was directly subjected to anionic ring opening polymerization of protected glycidol (grafting from approach). As a result dendritic solid support PS-D-LPG with linear polymeric chains containing functionalized ethylene glycol repeating units was developed. The Synthetic procedure is summarized in Scheme 1.

Hydroxyl terminated PS-D-OH resin (hydroxyl group capacity-1.3 mmol g^{-1}) was separately subjected to the most accepted anionic type polymerization of protected glycidol, in order to graft multivalent linear polymeric chains to the resin. To prevent branching, the hydroxyl group of glycidol was protected using ethyl vinyl ether according to the procedure described by Fitton et al. [40] The resulting ethoxyethylglycidyl ether was used for the surface initiated polymerization process. The hybrid support (PS-D-LPG) obtained was treated with aqueous HCl to remove the hydroxyl protecting group. The hydroxyl group capacity of the resulting resin (PS-D-DLPG) was determined by volumetric method using acetic anhydride [41] and it was found to be 1.5 mmol g^{-1}. The IR spectrum of the resin PS-D-OH and PS-D-LPG is given in Fig. 1. The band at 1100 cm^{-1} in the IR spectrum (PS-D-LPG) clearly indicated the linear polymer chain grafting from the resin and the final resin was yellow in colour.

EDAX spectrum of the dendritic solid support PS-D-LPG (Fig. 2) also supported the IR spectral results. The elemental composition of PS, PS-D-OH, PS-D-LPG are presented in Table 1. The sudden increase in oxygen content in the EDAX data of PS-D-LPG compared to PS and PS-D-OH also supported the polymer grafting.

The successful polymer grafting was further confirmed by CP-MAS ^{13}C NMR spectrum. The CP-MAS ^{13}C NMR spectrum of the resin PS-D-LPG is presented in Fig. 3.

In CP-MAS ^{13}C NMR spectrum of the resin PS-D-LPG, the sharp signal at 127.7 ppm and the signal at 145.7 ppm were attributed to aromatic carbons of phenyl ring in the polystyrene backbone. The broad signal at 70.6 ppm indicated the aliphatic carbons present in the back bone of linear polyglycidol chains. The peaks at 100.4, 62.6, 20.2, and 15.6 ppm were assigned to carbons present in ethoxyethyl protecting group. The signals marked at 45.6, and 40.3 ppm indicated the aliphatic carbon atoms present in the polystyrene support. The signal marked at 29.5



Scheme 1. Synthesis of the dendritic solid support PS-D-LPG.

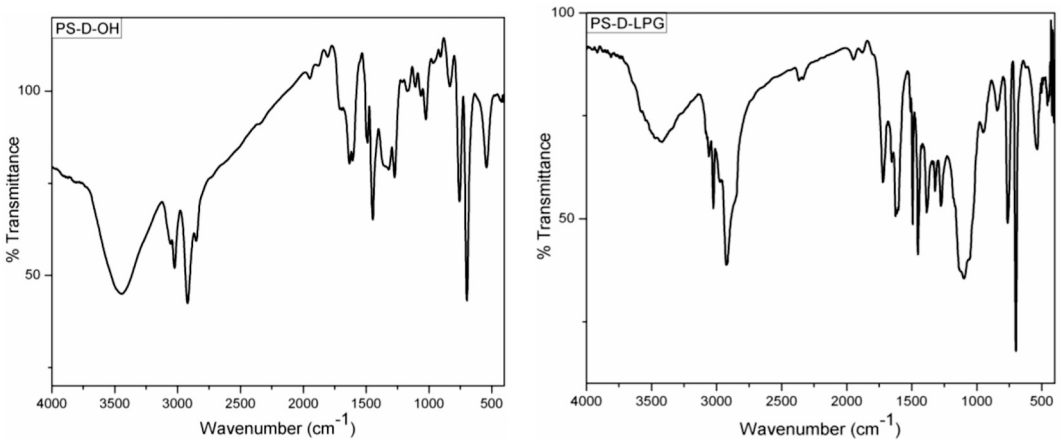


Fig. 1. IR spectra of various stages of PS-D-LPG synthesis.

ppm pointed out the bis-(3-hydroxypropyl)amine segment and the signals due to other carbon atoms present in bis-(3-hydroxypropyl)amine segment were found to be merged with various other peaks in the system.

Thermal stability of PS-D-LPG was studied by thermogravimetry (TG) analysis. The TG-DTG plots of PS, PS-D-OH and PS-D-LPG are given in Fig. 4.

The TG-DTG plot of PS showed the thermal degradation of polystyrene resin at 427 °C. In addition to the major weight loss above 400 °C, the TG-DTG plot of PS-D-OH showed an initial 10% weight loss at 247 °C and the weight loss was assigned to the degradation of bis-(3-hydroxypropyl)amine segment present in the resin. The TG-DTG plot of PS-D-LPG showed a major weight loss above 400 °C which indicated the degradation of polystyrene resin. The initial weight loss between 250 and 350 °C was attributed to the degradation of bis-(3-hydroxypropyl) amine segment followed by the degradation of linear polyglycidyl ether

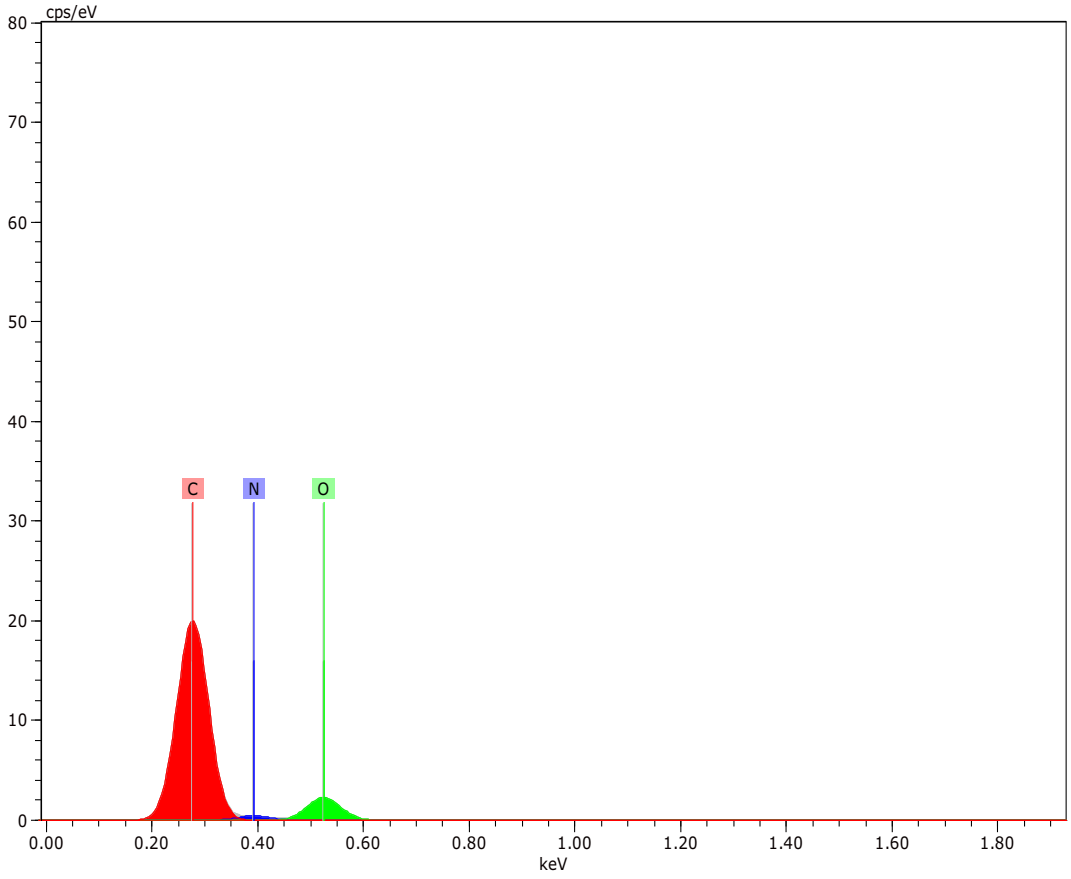


Fig. 2. EDAX spectrum of the dendritic solid support PS-D-LPG.

Table 1
Combined EDAX data.

Element	Mass % (PS)	Mass % (PS-D-OH)	Mass % (PS-D-LPG)
C, K	96.35	78.18	66.15
O, K	_	8.77	22.89
N, K	_	11.73	10.96
Cl, K	3.65	1.32	_
Total	100	100	100

chains. The hybrid support PS-D-LPG showed good thermally stability upto 250 °C. This observation also suggested the absence of adsorbed low volatile ethoxy ethyl glycidyl ether in the new support PS-D-LPG.

4.2. *Synthesis of dendritic solid support Het-PG via hypergrafting strategy*

In order to increase the loading capacity of the resin to a higher extent, anionic polymerization of glycidol was carried out on –OH terminated resin (PS-D-OH). The synthetic procedure is summarized in Scheme 2. Heterogeneous hypergrafting strategy was successfully

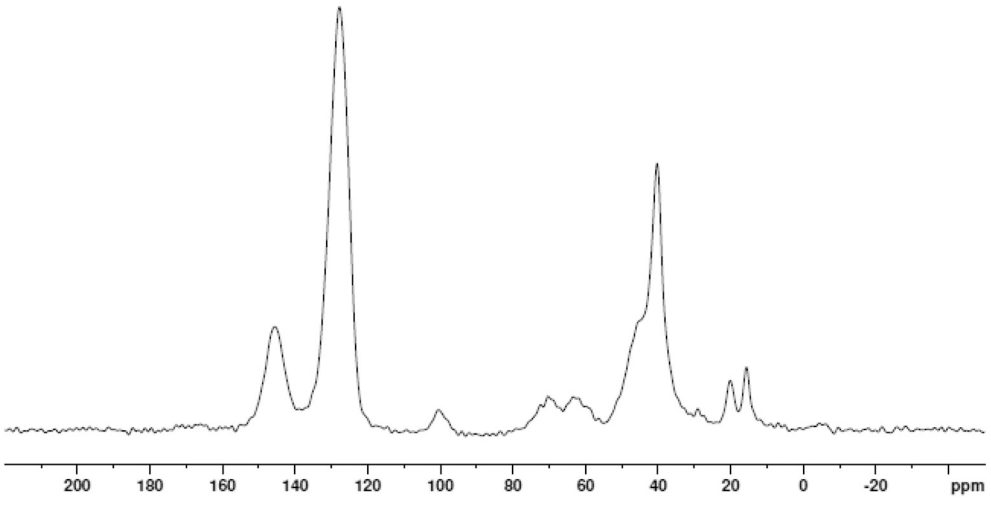


Fig. 3. CP-MAS ^{13}C NMR spectrum of the dendritic solid support PS-D-LPG.

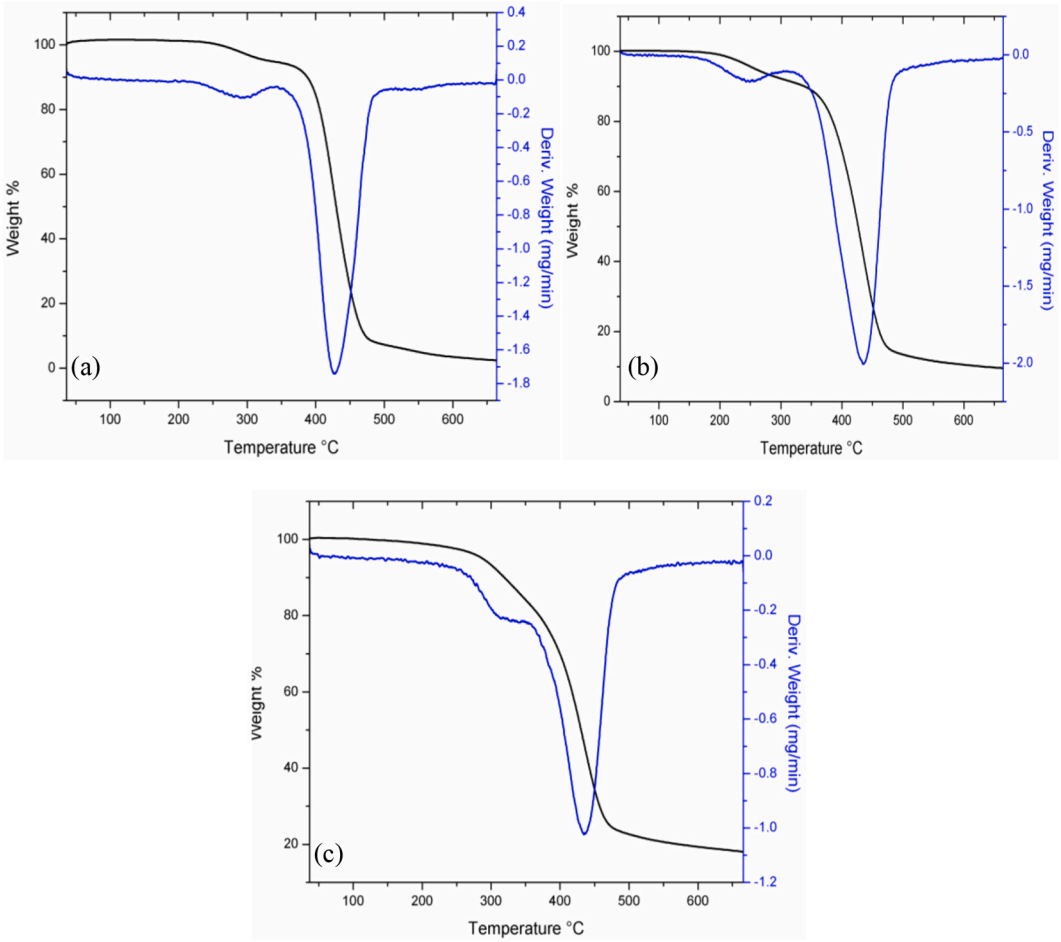


Fig. 4. TG-DTG plot of a) PS, b) PS-D-OH, c) PS-D-LPG.

employed for the synthesis of dendritic solid support Het-PG. Heterogeneous hypergrafting strategy was reported to be a versatile method for the development of dendritic solid support with high functional group capacity by a single step approach. This heterogeneous hypergrafting method avoided the previous synthesis/purification of the dendritic segment and the modification of dendritic segment necessary for the attachment to the surface. The dendritic polymer synthesis and its grafting to the surface of the resin took place simultaneously within a single step. The method also allowed the easy separation of soluble polyglycidol formed by the self polymerization of glycidol. In order to get high conversion during surface grafting, the complete metalation of the initiator (PS-D-OH) followed by very slow monomer addition method was used for the synthesis of dendritic solid support (Het-PG).

The six times increase in weight of final product after surface initiated anionic polymerization of glycidol on PS-D-OH indicated the hypergrafting of dense polyglycidol shell over polystyrene core. The resulting dendritic solid support, Het-PG represented a core-shell type support and showed good swelling capacity in polar solvents. Het-PG constitutes almost 20% polystyrene as core and 80% polyglycidol shell. The final resin was pale yellow in colour and the hydroxyl group capacity of the dendritic solid support Het-PG was determined to be 7 mmol g^{-1} by volumetric method using acetic anhydride [41]. Het-PG was found to be equivalent of supported higher generation dendrimer in terms of high functional group capacity and increased accessibility of functional sites in reaction medium. The dense polygycidol on Het-PG was found to provide a homogeneous like environment to bound substrates in polar reaction medium [39].

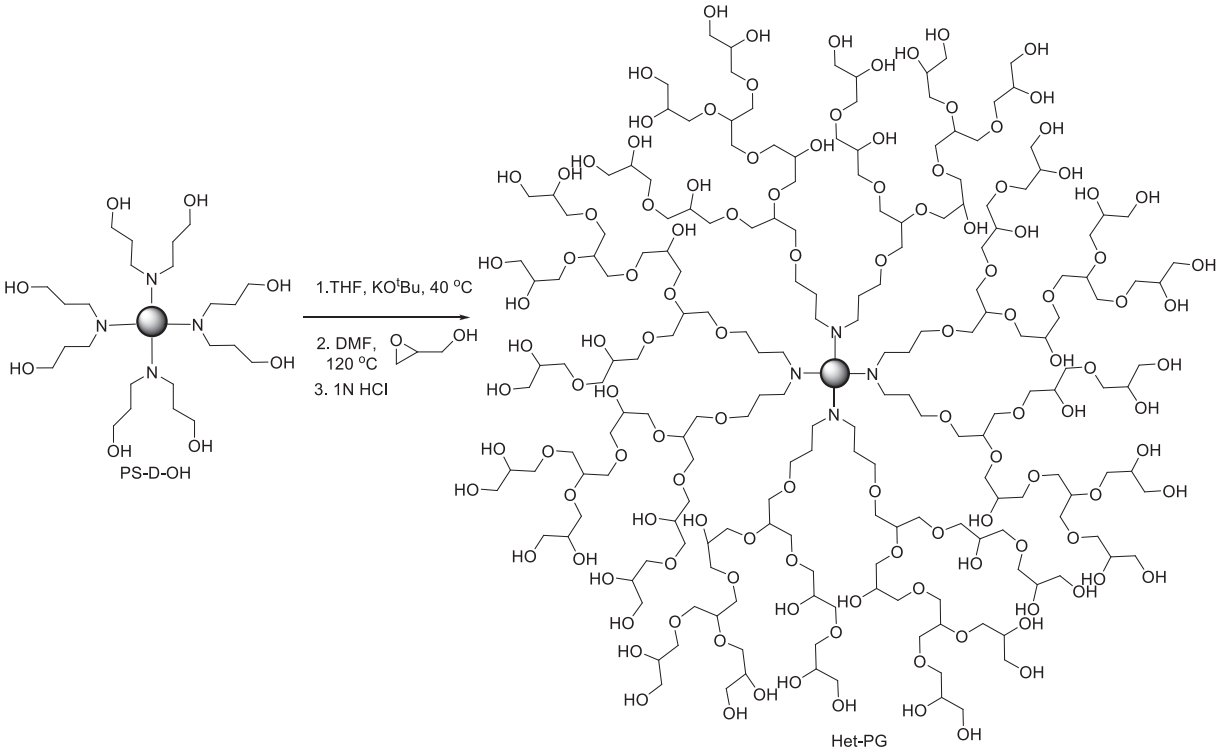
The details of the synthesis and characterization of dendritic solid support (Het-PG) have been published by our group [39]. The experimental procedure and scheme for the synthesis of Het-PG has been given in supporting information (SI-Scheme 2). The sharp bands at 1100 cm^{-1} (ether linkage), and 3400 cm^{-1} (hydroxyl groups) in the IR spectrum of Het-PG, and the sudden increase in oxygen content in EDAX data of Het-PG etc. supported the formation of Het-PG via hypergrafting. The signals in the range 60–80 ppm in CP-MAS ^{13}C NMR spectrum of Het-PG also indicated the polyglycidol graft. Characterization data of Het-PG has been given in supporting information (SI- Fig. 2, Figs. 3, 4).

Thermal stability of Het-PG was studied by thermogravimetry (TG). The TG-DTG plot of Het-PG is given in Fig. 5. Het-PG exhibited an excellent thermal stability upto 350 °C. The DTG plot of Het-PG showed a narrow peak of 100% weight loss at 426 °C. Since 80% by weight of the dendritic solid support Het-PG is dendritic polyglycidol, the single narrow degradation peak of 100% weight loss at 426 °C suggested the relatively similar thermal stability of polystyrene and dendritic polyglycidol.

4.3. Synthesis of dendritic solid support PS-D-OXE via hypergrafting strategy

In order to get a high loading dendritic solid support with uniform terminal hydroxyl functional groups, ring opening polymerization of 3-methyl-3-hydroxymethyl oxetane was carried out on hydroxyl terminated resin (PS-D-OH). Both cationic and anionic polymerization of 3-methyl-3-hydroxymethyl oxetane was carried out for hypergrafting on the resin. The best result was obtained for cationic polymerization method and the synthetic procedure is summarized in Scheme 3.

The anionic polymerization of 3-methyl-3-hydroxymethyl oxetane was carried out using potassium tertiary butoxide and 18 crown 6, at



Scheme 2. Synthesis of the dendritic solid support Het-PG.

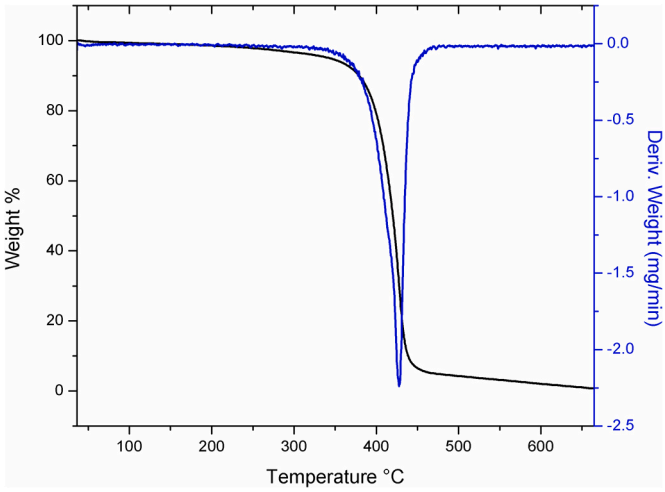


Fig. 5. TG-DTG plot of the dendritic solid support Het-PG.

elevated temperature 180 °C whereas the cationic polymerization of 3-methyl-3-hydroxymethyl oxetane was effective for hypergrafting on the resin at room temperature in the presence of BF_3-etherate. Polymer grafting was easily identified by the presence of the bands corresponding to ether linkage (1100 cm^{-1}) and hydroxyl group (3430 cm^{-1}) in the IR spectrum of the resin PS-D-OXE as shown in Fig. 6. The increased oxygen content in the EDAX data of PS-D-OXE compared to PS-D-OH also indicated the polymer grafting on the resin. EDAX spectrum and EDAX data are given in Fig. 7.

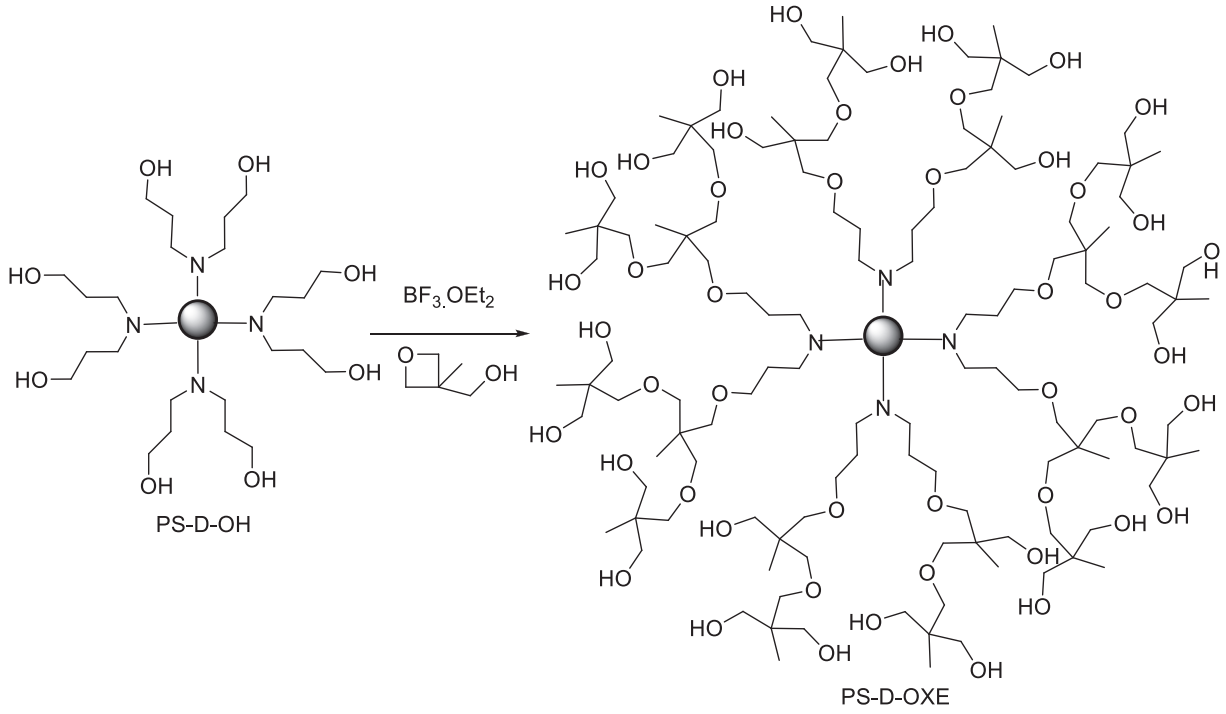
The successful polymer grafting was further confirmed by CP-MAS ^{13}C NMR spectrum. In CP-MAS ^{13}C NMR spectrum of the resin, PS-D-OXE as shown in Fig. 8, the sharp signal at 127.9 ppm and the signal at 145.9 ppm were attributed to the aromatic carbons of phenyl ring in the polystyrene backbone. The broad signal at 74.5 ppm, and the sharp signal at 18.25 ppm due to free methyl groups clearly indicated the hypergrafted polyoxetane. The broad peak at 41.8 ppm may be due to the combined signal of the aliphatic carbon atoms present in the polystyrene support and the tetra-substituted carbons at the periphery of dendritic polyoxetane graft. The signal at 67.2 ppm was assigned to the carbon atoms near to terminal hydroxyl group in the polyoxetane graft and also the peak represented the carbon atoms near to oxygen in bis-(3-hydroxypropyl)amine segment. The signal marked at 35.5 ppm pointed out the tetrasubstituted carbon at the branching segment. The signals due to other carbon atoms were found to be merged with various other peaks in the system.

The final resin was yellow in colour and the hydroxyl group capacity of the hybrid support PS-D-OXE was determined to be 1.6 mmol g^{-1} by volumetric method using acetic anhydride [41]. The thermal stability of PS-D-OXE was studied by thermogravimetry (TG) analysis. The TG-DTG plot of PS-D-OXE resin is given in Fig. 9. PS-D-OXE exhibited excellent thermal stability upto 350 °C. The minor weight loss above 500 °C indicated the polyoxetane graft. The dendritic polyoxetane graft was not destructed upto 350 °C. This observation suggested the comparable thermal stability of polystyrene and polyoxetane.

4.4. Solid phase peptide synthesis using dendritic solid support

All the hybrid supports exhibited excellent thermal stability and improved accessibility of functional sites compared to polystyrene resin. The swelling capacities of all hybrid supports in water are presented in Table 2. The dendritic solid supports combined the benefits of easy recovery, high functional group density, increased accessibility of functional sites, etc. These supports can be easily synthesized in large quantities and can be separated from the reaction mixture by standard laboratory techniques.

Eventhough the same amount of different cyclic monomers (same number of moles of each monomer for 1 g of PS-D-OH resin) and same temperature conditions were used in grafting reaction, grafting efficiency was different in the synthesis of each hybrid support. The different grafting efficiency may be due to the difference in the nature of monomers. The functional group capacity per gram of resin (determined



Scheme 3. Synthesis of the dendritic solid support PS-D-OXE.

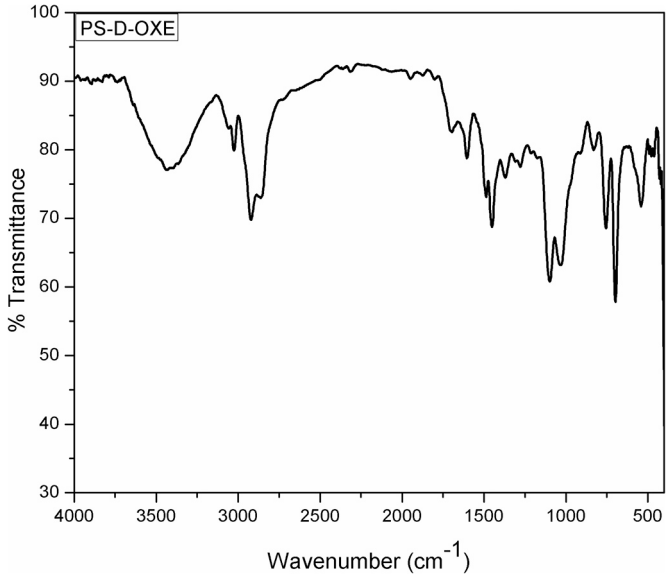


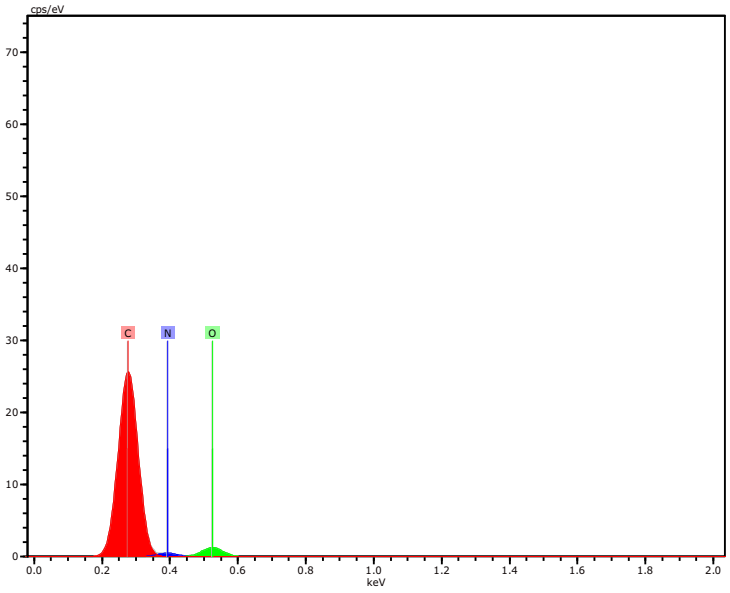
Fig. 6. IR spectrum of the dendritic solid support PS-D-OXE.

by volumetric method) and weight of resin after hypergrafting reaction indirectly gave information about the amount of monomer units grafted to the resin.

The maximum grafting efficiency was obtained for the dendritic solid support Het-PG. Among the dendritic solid supports, Het-PG showed the maximum functional group capacity (7 mmol g^{-1}). The special attraction of Het-PG was mainly due to its similarity to supported higher generation dendrimers. Such a high loading capacity has not been reported in any other dendritic solid supports via hypergrafting strategy. Het-PG represented a highly loaded dendritic solid support with wide range of solvent compatibility necessary to facilitate a more or less solution like chemistry of bound substrates. The very high functional group loading, water compatibility, thermal and mechanical stability, the special core-shell type structure with a dense hydrophilic polyglycidol shell (80% by weight) which can be extended out in polar reaction media etc., are the beneficial features of Het-PG, which make Het-PG a promising solvent like dendritic solid support for the solid phase organic synthesis.

Generally, low loading solid supports were found to be efficient for solid phase peptide synthesis. Also the efficiency of solid phase peptide synthesis depended upon swelling property of the solid support. Since most of the functional groups are at the periphery of large polyglycidol shell (80% by weight of Het-PG) which can be extended out in polar reaction media, the concept of swelling is not much important in the case of the newly developed solid support Het-PG. In suitable solvents the large polyglycidol shell is expected to provide a solution like environment to bound substrates. The improved catalytic activity of NHC-Pd complex immobilized on Het-PG has been recently reported by our group [39]. This also suggests the solution like environment around the substrate bound to the dendritic solid support Het-PG. So Het-PG is a promising support which can extend the general concept of solid phase peptide synthesis using low loading solid support (0.5–1 mmol g^{1}) to solvent like high loading hybrid solid support. Swelling properties of Het-PG hybrid resin in various solvents are summarized in Table 3. Considering the increased accessibility of functional sites in the reaction medium, even at high loading level, the applicability of Het-PG for solid phase peptide synthesis was investigated.

Synthetic efficiency of the support Het-PG was studied by carrying out the solid phase peptide synthesis of small model peptide, Ala-Ala-Gly. This peptide sequence is the fragment of ribosomal protein of *E. coli* bacteria. Though it is a very small peptide, it has a strong tendency to form β sheet structure. In this respect, the synthesis of Ala-Ala-Gly using solid phase methodology is really challenging. β sheet stabilizing potential value (<SP_β>) of the model peptide, Ala-Ala-Gly is almost 5.0 according to literature reports [42]. The standard Boc strategy was selected for amino group protection in the solid phase peptide synthesis. The most common N,N′- dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) was used as coupling agents. Final peptide cleavage from the solid support was carried out using trifluoroacetic acid (TFA).



Element	Mass %
C, K	74.39
O, K	13.23
N, K	12.38
TOTAL	100

Fig. 7. EDAX spectrum and EDAX data of the dendritic solid support PS-D-OXE.

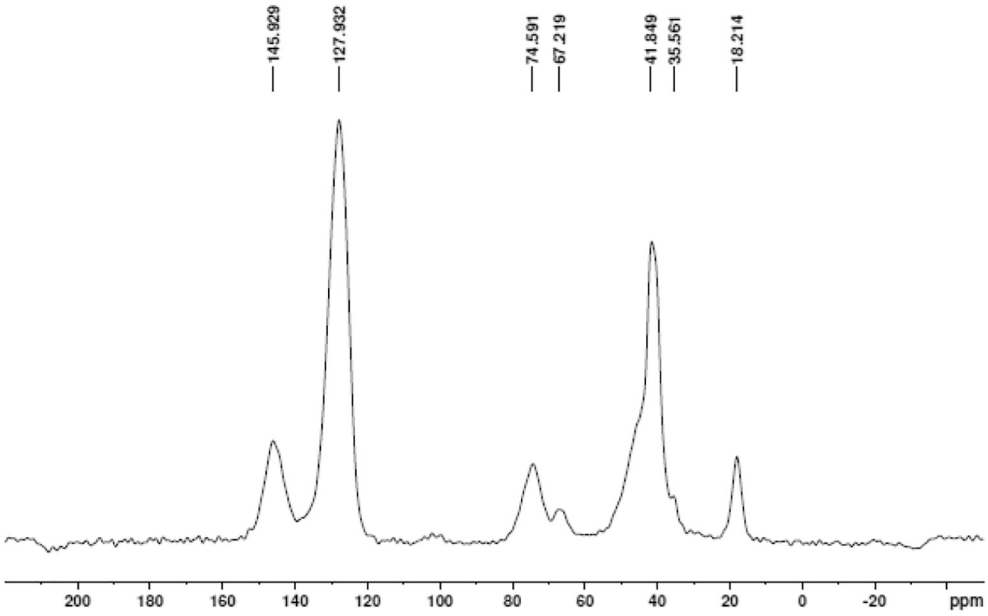


Fig. 8. CP-MAS ^{13}C NMR spectrum of the dendritic solid support PS-D-OXE.

In order to use a support for solid phase organic synthesis, it should be chemically stable throughout the synthetic steps and the product should be selectively cleaved from the support using specific reagents. Solid phase peptide synthesis using Boc strategy is associated with strong acidic condition which may affect the solid support. So the chemical stability of the dendritic solid support Het-PG in neat trifluoroacetic acid, the reagent generally used to cleave the peptide from support was studied by recording the IR spectrum of the respective resin after TFA treatment and comparing it with IR spectrum of original support. The solid support Het-PG was treated with TFA for 24 h, and IR spectra were recorded after proper washing. The comparable IR spectra of the resin before and after TFA treatment showed that the solid support Het-PG was chemically stable throughout the period of treatment.

Primary amino acid attachment to the hydroxyl terminated resin was achieved using 5 mmol excess of the Boc-protected amino acid at the peptide C-terminal along with N,N′-diisopropylcarbodiimide(DIC), HOBt and 4-dimethylaminopyridine (DMAP). The presence of a sharp band corresponding to ester carbonyl stretching in the IR spectrum of Boc-glycine attached Het-PG (Het-PG-PA) as shown in Fig. 10, indicated the first amino acid attachment to the solid support. The first amino acid attachment was found to be very low compared to the total functional group capacity of Het-PG support and it was clear from the comparatively low increase in weight of solid support than expected after the first amino acid attachment using DIC, HOBt &DMAP. The strong hydrogen bonding interaction present in Het-PG support may be the reason for the relatively low loading of first amino acid on Het-PG. The initial loading of amino acid was found to be 1.07 mmol g^{-1}. To avoid the hydrogen bonding interaction present in Het-PG support, azide terminated dendritic solid support Het-PG-N_3 was developed. The details of the synthesis and characterization of each stage of the development of azide terminated dendritic solid support Het-PG-N_3 has been published by our group [39]. The hydroxyl groups of Het-PG were activated using

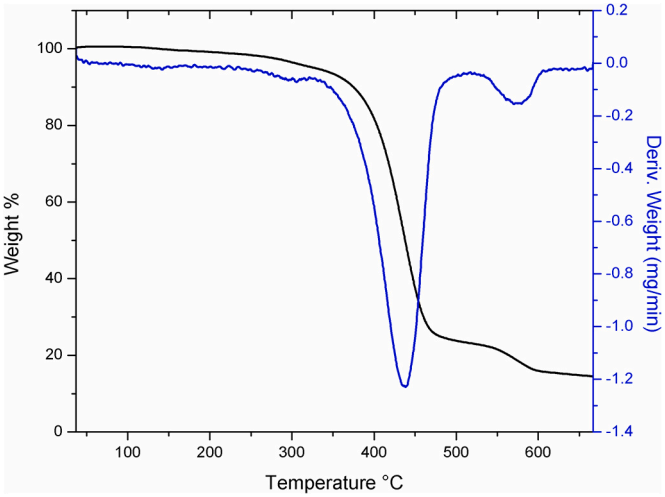


Fig. 9. TG-DTG plot of the dendritic solid support PS-D-OXE.

Table 2
The swelling capacities of different hybrid supports in water.

Hybrid resin	PS-D-LPG	Het-PG	PS-D-OXE
Swelling capacity in water	2 mL g^{-1}	7 mL g^{-1}	1.5 mL g^{-1}

Table 3
Swelling properties of Het-PG hybrid resin in various solvents.

Resin	Swelling capacity (mL g^{-1})		
	CH_2Cl_2	DMF	H_2O
Het-PG	4 mL g^{-1}	4.5 mL g^{-1}	7 mL g^{-1}

methane sulfonyl chloride in pyridine. The resulting Het-PG-OMS was easily transformed in to its azide terminated form Het-PG-N_3 using excess sodium azide. The detailed scheme, experimental procedures and IR spectrum of each stage for the synthesis of azide terminated dendritic solid support Het-PG-N_3 from Het-PG has been given in supporting information (SI-Scheme 3, SI- Fig. 5).

The functional group capacity of all synthesized hybrid supports and some reported multifunctional polymer grafted high loading hybrid supports are summarized in Table 4.

In order to improve the first amino acid attachment to the solid support, propargyl ester of Boc protected first aminoacid glycine was prepared and the propargyl ester of Boc-glycine was attached to azide terminated Het-PG-N_3 support (7 mmol g^{-1}) using copper catalyzed click chemistry. The Boc deprotection protocol and DCC&HOBt mediated coupling was repeated for subsequent aminoacid attachment of the model peptide Ala-Ala-Gly. The synthetic procedure is summarized in Scheme 4.

The final peptide was cleaved from the support using TFA/thioanisole in the volume ratio (10:1). The peptides were finally precipitated using chilled ether and washed thoroughly with cold ether to remove the scavengers. The peptide was dried. The resin was subjected to a second cycle of cleaving to ensure complete removal of peptide from the support.

The first amino acid attachment was confirmed by the presence of a band corresponding to ester carbonyl stretching in the IR spectrum of Boc-glycine propargyl ester attached Het-PG-N_3 (Het-PG-N_3-PA) as shown in Fig. 10. The absence of a band at 2100 cm^{-1} due to azide group clearly indicated the complete replacement of azide group by propargyl ester of Boc-glycine via triazole linkage. The considerable increase in weight of solid matrix after click chemistry also suggested the comparatively better loading of first aminoacid. The initial loading of amino acid was found to be 2.58 mmol g^{-1}. The weight of solid matrix after click reaction and IR spectrum, suggested the availability of the whole functional sites of the support Het-PG-N_3 for initial loading of amino acid. The click reaction was found to be an efficient method for producing better initial loading of aminoacid on Het-PG and hence the high functional group capacity of solvent like dendritic solid support Het-PG was explored for solid phase peptide synthesis. The double coupling method with 2.5 equivalents of aminoacid and DCC&HOBt as coupling

Table 4
The functional group capacity of high loading hybrid supports.

Hybrid supports	Functional group capacity	Application
1. G_4 Tris based dendronized CutiCore resin [17]	1.7 mmol g^{-1}	Solid phase peptide synthesis
2. PEGylated G_2 dendronized polystyrene resin [21–23]	0.3–0.5 mmol g^{-1}	Solid phase peptide synthesis
3. PS-PEGA [25]	0.5–1.2 mmol g^{-1}	Solid phase peptide synthesis
4. ROMP-Spheres [26]	3.03 mmol g^{-1}	Solid phase organic synthesis
5. PS-g-PG [28]	4.3 mmol g^{-1}	Solid phase organic synthesis
6. PS-D-DLPG	1.5 mmol g^{-1}	New support
7. PS-D-OXE	1.6 mmol g^{-1}	New support
8. Het-PG	7 mmol g^{-1}	New support
9. Het-PG-N_3	7 mmol g^{-1}	New support

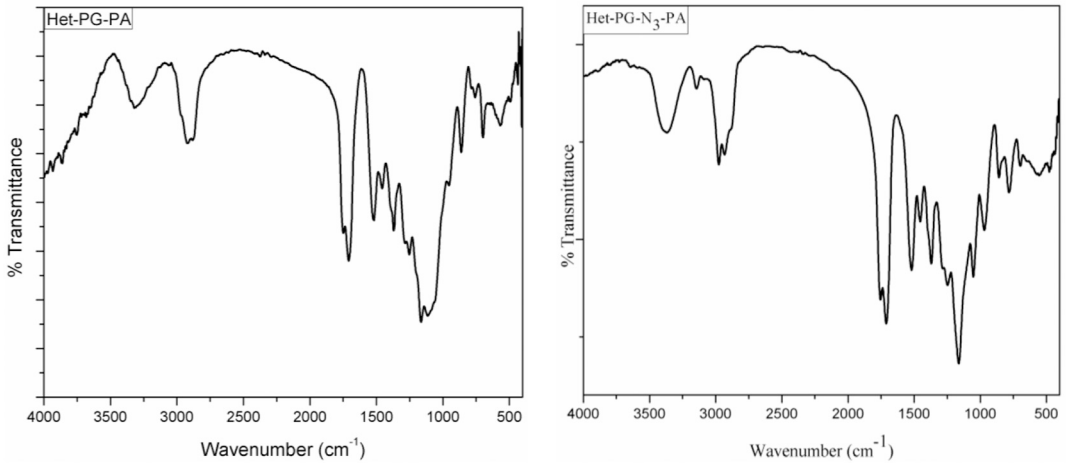
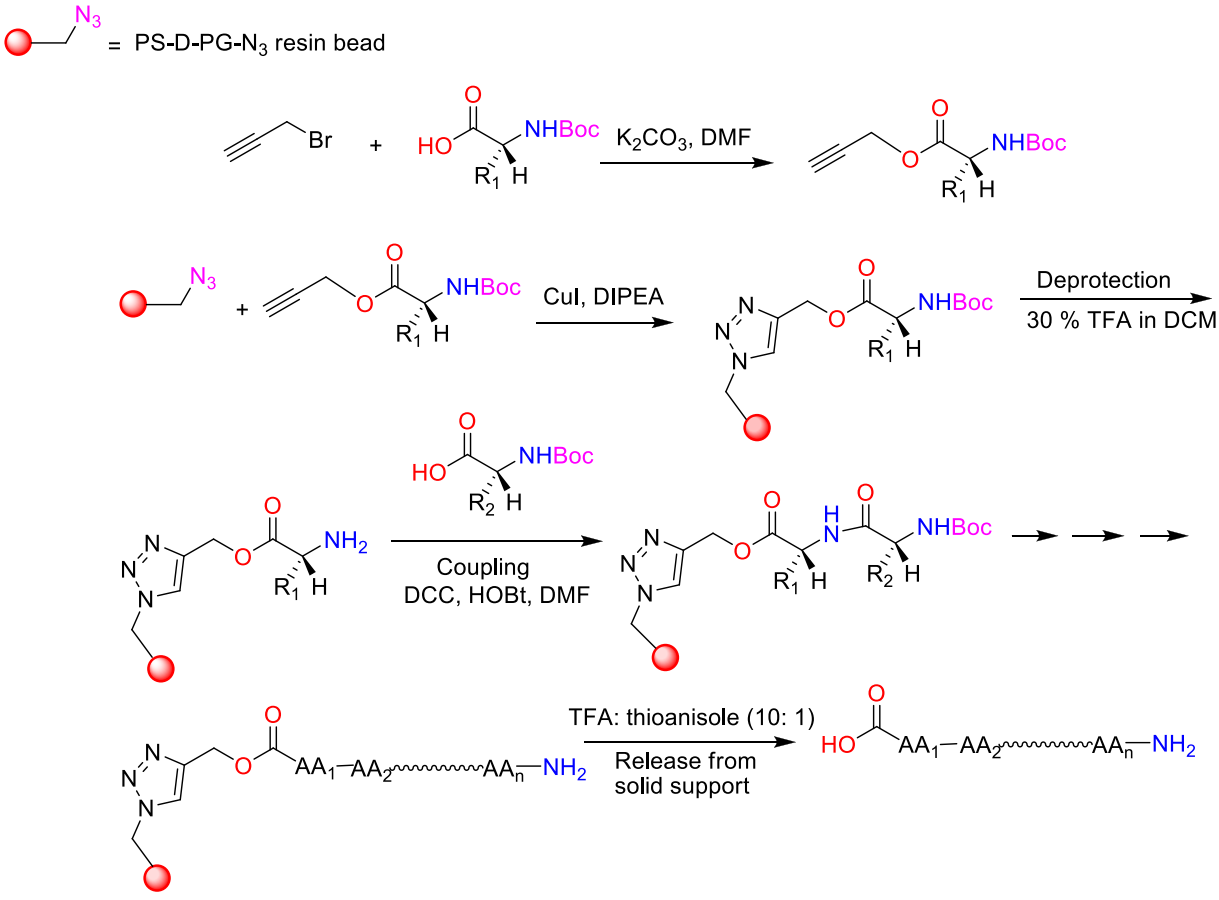


Fig. 10. IR spectrum of 1st protected amino acid attached Het-PG resin and Het-PG-N_3 support.



Scheme 4. Solid phase peptide synthesis using Het-PG-N_3.

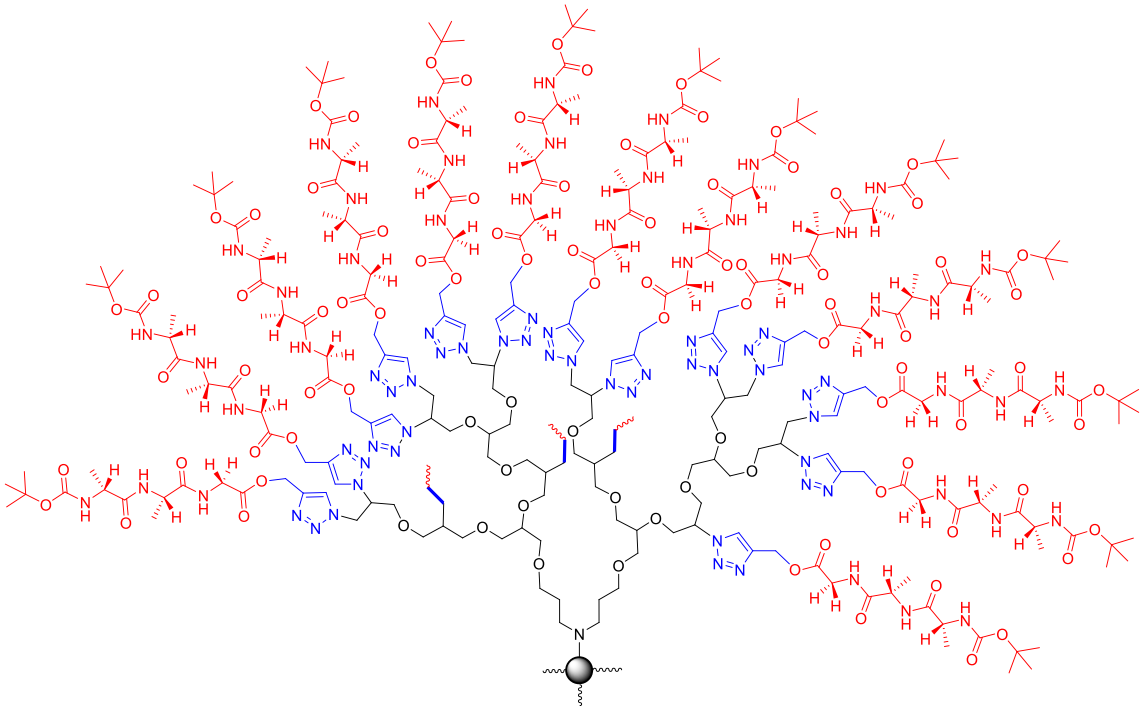


Fig. 11. Peptide bound Het-PG-N_3.

agent for a period of 1 h was repeated for subsequent addition of aminoacid in the desired sequence. Each amino acid attachment and Boc-deprotection was monitored using ninhydrin test. The structure of peptide bound Het-PG-N_3 is given in Fig. 11.

The structure of peptide bound Het-PG-N_3 was studied by solid state ^{1}H NMR and ^{13}C NMR spectrum. The solid state ^{1}H NMR and ^{13}C NMR spectra of peptide bound Het-PG-N_3 are presented in Figs. 12 and 13 respectively. Due to the presence of large number of hydrogen atoms in the polymeric structure, the solid state ^{1}H NMR spectrum of peptide bound Het-PG-N_3 did not give much information about the detailed structure of peptidyl resin. But the sharp signals marked at 1.45 ppm, 1.29 ppm, and 1.20 ppm in the solid state ^{1}H NMR spectrum clearly pointed out the methyl hydrogen of alanine and tertiary butyloxycarbonyl protection of last aminoacid in the resin supported peptide segment respectively.

The solid state CP-MAS ^{13}C NMR spectrum of peptide bound Het-PG-N_3 resin gave an insight to the structure of peptidyl resin (Fig. 13). In the spectrum, the peak at 172.3 ppm indicated the combined signal of carbonyl carbon of amide and ester linkage in peptidyl resin. The sharp signal at 28.6 ppm and the signal at 156.4 ppm were assigned to methyl carbon and carbonyl carbon of tertiary butyloxycarbonyl protection of last amino acid in the peptidyl resin. The signal at 17.1 ppm pointed out the methyl group of alanine in the synthesized Ala-Ala-Gly peptide. The signal at 41.0 ppm indicated the methylene carbon of first aminoacid glycine in the peptide fragment. The combined signals due to aromatic carbon of crosslinked polystyrene and carbon atoms present in triazole linkage appeared at 126.3 ppm, 147.8 ppm and 143.3 ppm. The signals at 143.3 ppm clearly indicated the triazole carbon attached to the peptide fragment. The peaks at 71.1 ppm and 79.1 ppm were attributed to the combined signal of carbon atoms near to oxygen in polyglycidol and the tertiary carbon present in Boc protection of last amino acid. The broad peak at 56.1 ppm was assigned to the carbon atoms near to triazole ring. The signal due to aliphatic carbon atoms of polystyrene was found to be merged with the peaks due to alanine C-H group and the carbon near to nitrogen in the bis(3-hydroxypropyl amine) segment. The combined signal appeared at 50.1 ppm.

Final peptide was cleaved from the support using TFA and it was purified. The purity of the peptide cleaved from Het-PG-N_3 was analysed using HPLC. HPLC analysis was carried out using C-18 column. Water (0.1% TFA): CH_3CN (0.1% TFA) - (1:1 *v*/v) was used as mobile phase with a flow rate of 0.5 mL min^{-1}. The detector wavelength was set as 210 nm. The HPLC profile (Retention time Rt-18) and ESI mass spectrum of peptide synthesized are presented in Fig. 14.

In the HPLC profile, the sharp peak at retention time 4.9 min may be due to the presence of dissolved air in the solvent used for preparing the peptide sample solution. The peak at retention time (Rt)- 18 min indicated the desired peptide. The HPLC profile showed the considerable purity of synthesized peptide even in the crude state itself. Similar to the conventional solid phase small peptide synthesis using Merrifield resin, the peptide synthesized using Het-PG showed considerable purity. The overall yield was found to be 49%. Still the amount of peptide synthesized from 1 g of Het-PG-N_3 (7 mmol g^{-1}) was higher than that expected from 1 g nondendritic Merrifield resin of functional group capacity 1 mmol g^{-1}. The relatively low yield may be attributed to the loss of fine peptidyl resin powder in each consecutive cycle as being attached to the filter paper used in standard manual multistep synthesis.

5. Conclusion

Three novel dendritic solid supports with high functional group capacity and increased accessibility of functional sites in the reaction medium were synthesized and characterized. Among the hybrid supports, the dendritic solid support Het-PG was found to be a promising solvent like high loading support, which combined the benefits and minimized the drawbacks related with solid phase synthesis. Using Het-PG, an attempt was made to extend the concept of solid phase peptide synthesis using low loading solid support to solvent like high loading dendritic solid support. Het-PG was studied as high loading support for solid phase peptide synthesis. Synthesis of a small model peptide Ala-Ala-Gly with $<SP_\beta>$ almost 5.0, using the high loading dendritic solid support Het-PG-N_3 was demonstrated manually under standard laboratory conditions using the common Boc-strategy and HOBt &DCC as coupling agent. Using click chemistry, the high loading capacity of solvent like dendritic solid support Het-PG-N_3 was explored in the solid phase peptide synthesis. The yield in the case of Het-PG-N_3 is a progressive result. By fine tuning the synthetic steps, the yield can be modified. Due to biocompatibility and high swelling capacity in water, the new dendritic solid support Het-PG offers extended applications for N-carboxyanhydrides based water mediated peptide synthesis and other multistep synthesis of biologically relevant molecules in protic solvents.

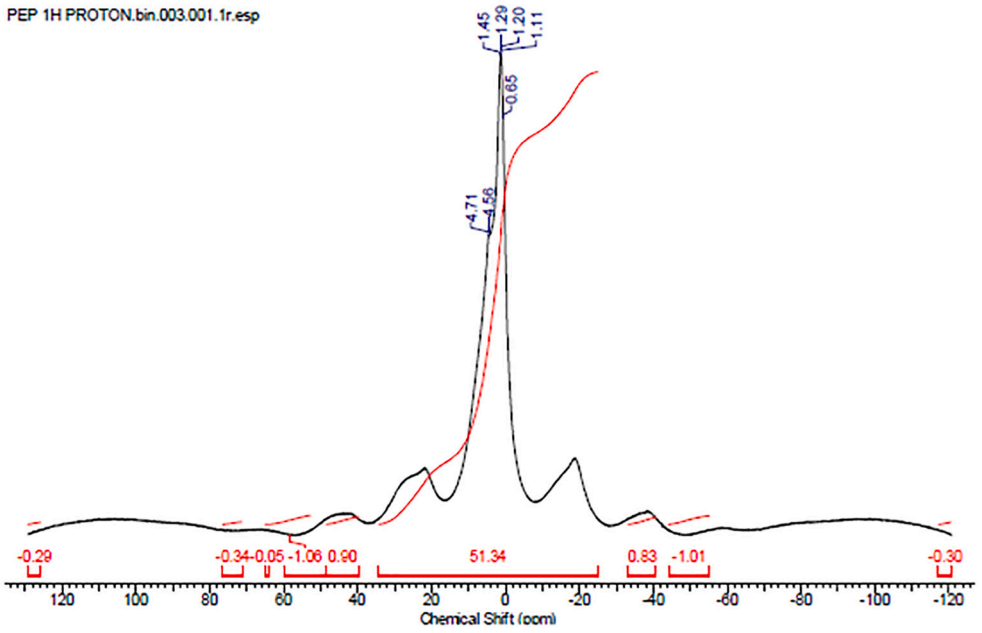


Fig. 12. Solid state ^{1}H NMR spectrum of peptide bound Het-PG-N_3 resin.

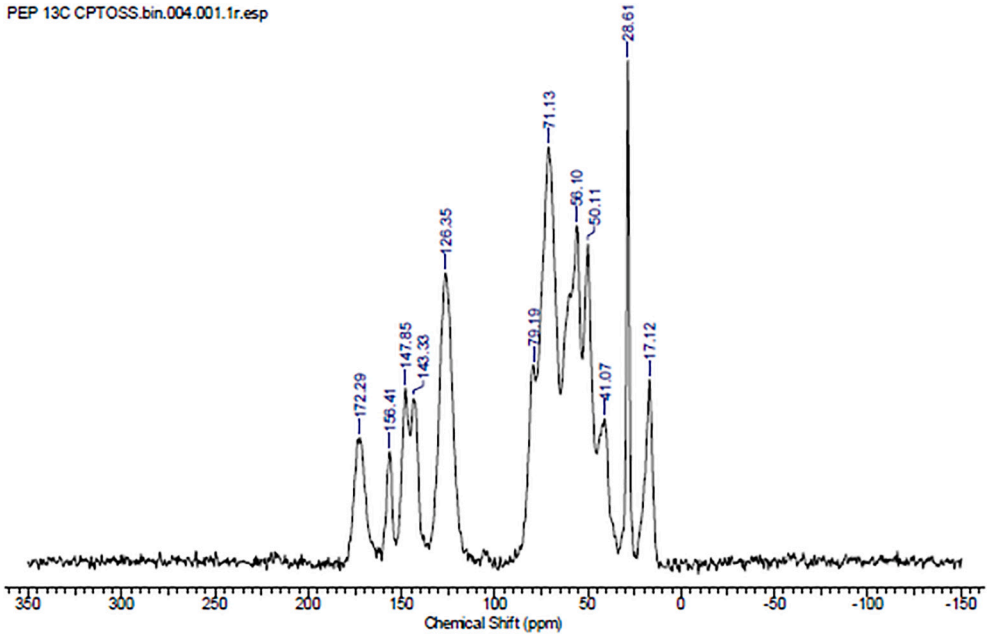


Fig. 13. Solid state CP-MAS ^{13}C NMR spectrum of peptide bound Het-PG-N_3 resin.

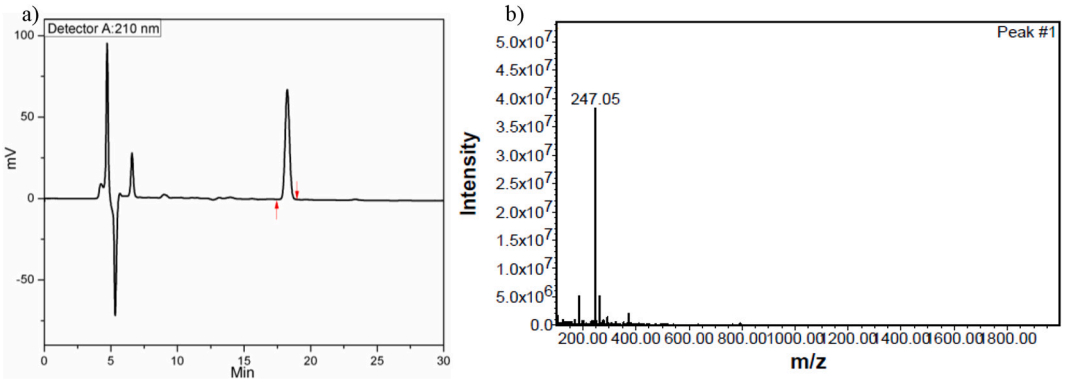


Fig. 14. a) HPLC profile and b) ESI mass spectrum of the peptide synthesized using Het-PG-N_3 support.

Data availability

The raw data required to reproduce these findings are included in the article.

Author statement

All authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been submitted to or published in any other publication.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

IR spectra, EDAX spectra, CP-MAS ^{13}C NMR spectra of some modified solid supports as appendix.

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