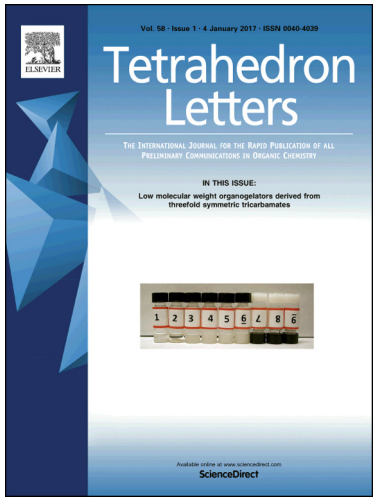

Multifunctional ionic liquid-bound polystyrene resin with high loading capacity as support in solid-phase peptide synthesis

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Multifunctional ionic liquid-bound polystyrene resin with high loading capacity as support in solid-phase peptide synthesis

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ABSTRACT

Polystyrene resin-bound ionic liquids (PSILs) with high loading capacities were prepared by immobilizing multifunctional ionic liquids (ILs) on modified polystyrene (PS) resin and used in the solid phase peptide synthesis. Introduction of hydrophobic anions and functional side chain containing ILs resulted in high yield (82-98%) and purity (92-98%) of the synthesized peptides. The coupling kinetic studies of the first and second amino acids to the PSILs were performed to investigate the effect of IL functionalization on PS supports.

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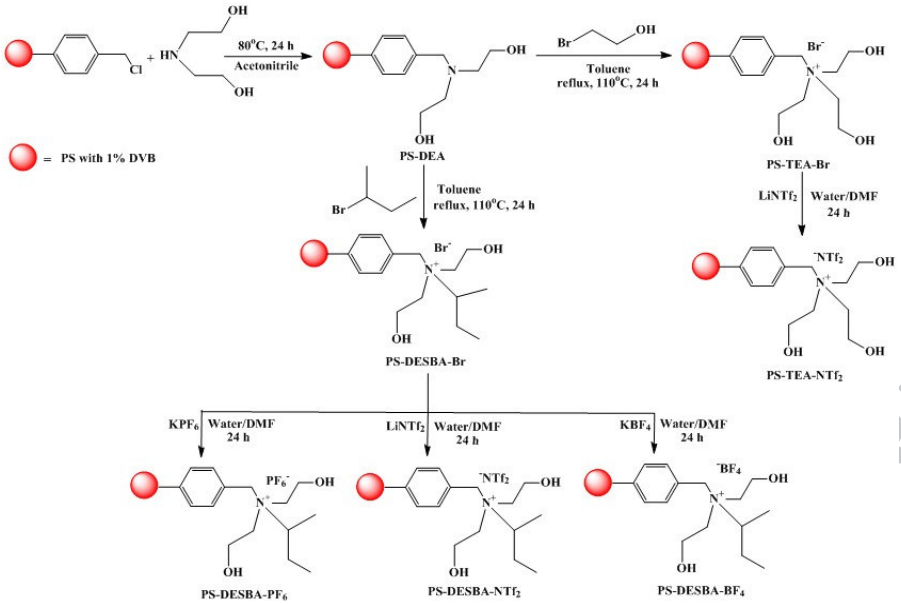
With the invention of a number of commercially important peptide-based pharmaceutical products as well as synthetic peptides, the innovative synthesis of peptides have gained a tremendous interest among the researchers in last few years. With the decade-long persisting problems in the field of peptide manufacture, the best method till date is still the solid-phase peptide synthesis (SPPS) prescribed back in 1963.[1,2] Several innovative methodologies are also reported in last few years in an attempt to get rid of the persisting problems in this field like low loading capacity of the available solid supports, separation problems in the homogeneous phase synthesis, poor kinetics of the SPPS, energy and cost-inefficient enzyme mediated processes, etc.[3] Most of these methods suffer from the disadvantages like low loading capacity, poor industrial scalability, costly starting materials, etc.[4] Polystyrene (PS) resins are most commonly used resin because of their good swelling property in various solvents and good chemical and mechanical stability. However, the hydrophobic matrix of these typical solid-phase resin supports leads to problems like aggregation of the growing peptides and poor diffusion of the amino acids and hence, retarded kinetics.[4] Hence, to improve the swelling properties of the hydrophobic supports, sustainable flexible supports with hydrophilic groups are to be developed.

Recently, ionic liquids (ILs) were reported as homogeneous phase soluble support for the C-terminal peptide synthesis.[5] Functionalized ILs were also reported as solvents in synthesis of peptides with difficult sequences with high yields.[6] The use of ILs in the homogeneous phase resulted in more controlled chain growth without any puckering or agglomeration, no racemization and controlled solubility of the peptides.[5,6] However, these methods suffer from the disadvantages like poor recyclability of the ILs used as homogeneous support, poor kinetics imparted by viscous ionic liquids along with inherent homogeneous phase problems like poor industrial scalability, solubility issues for larger peptides, etc.

Hence, to address these issues, efficiency of imidazole-based alkylamine functionalized PS resin-bound ILs (PSILs) were investigated using the inherent advantages imparted by ILs in solid-phase C-terminal peptide synthesis approach.[7] A new methodology using PSILs with excellent swelling property were successfully used for the synthesis of oligo and polypeptides. However, the reported PSILs suffered with issues like low loading capacity, requirement of additional linker, etc. Hence, the methodology can be further explored to improve the loading capacities, optimize the reaction conditions, improvement of the kinetics, substitution of toxic imidazole components from the support, etc.

Recently, diethanolamine (DEA)-based PSILs with high loading capacities were reported earlier for the chemical fixation of CO_2 with very high efficiency.[8] The immobilization of the –OH group containing functionalized ILs on PS changes the flexibility of the resin moiety. In this study, DEA-based ILs were supported on the PS resin and used as support in C-terminal peptide manufacture process. The anion metathesis of the ILs led to the formation of a series of ILs and their effect on the coupling kinetics of the amino acid was investigated. The synthetic efficiency of the PSILs on the peptide manufacture cycle was also demonstrated by the synthesis of oligopeptides.

To improve the swelling properties of the support, 1% divinylbenzene containing PS resins were used. The loading of diethanolamine on the PS resin was achieved by the substitution of the –Cl group of chloromethyl PS (PS-Cl) with the N-atom to obtain PS-DEA. The synthesized PSILs as shown in **Scheme 1**, were composed of an additional spacer to stop additional inter-chain interactions among the amino acids of the growing peptide chains. Reaction with 2-butyl bromide led to the formation of bromide containing PSIL as prescribed in literature.[8] Further, substitution of the Br^- anion with entities like PF_6^-, BF_4^- and NTf_2^- was achieved by the anion metathesis with their corresponding Li or K salts in a 1:1 mixture of water and DMF. A similar triethanolic IL (as shown in **Scheme 1**) was synthesized by addition of 2-bromoethanol group to PS-DEA followed by substitution of the bromide anion with NTf_2. FT-IR spectroscopic studies were firstly carried out with PS-Cl, PS-DEA and pure DEA. Prior to analysis the samples were dried over phosphorus pentoxide under reduced pressure at 70°C for 24 h. The loading of DEA on the PS-Cl was confirmed by the presence of a strong peak around 3400-3450 cm^{-1} in both DEA and PS-DEA as shown in **Suppl. Figure 1**. Complete DEA loading on the PS-Cl by removal of –Cl groups was confirmed by the absence of C-Cl bond stretching (1265 cm^{-1}) in the FT-IR spectrum of the resin, PS-DEA. The loading% of the ILs on PS resin was determined by the nitrogen content estimated from elemental analysis.



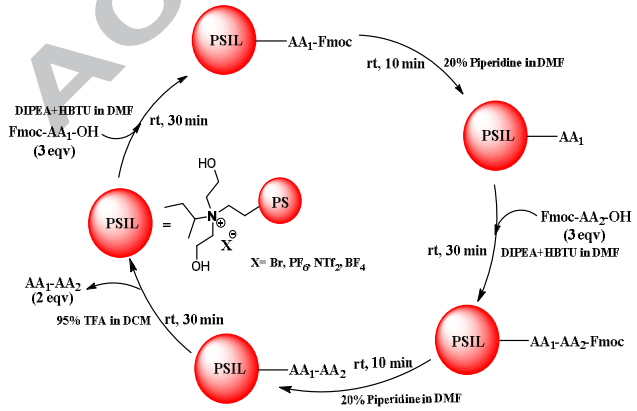
Scheme 1. Synthetic pathway of the PSILs.

SEM images and the EDX data of the pure PS and PSILs are shown in **Suppl. Figure 2**. The loading of ILs on the PS does not alter the morphology of the surface as evident from the SEM images. The anion metathesis of the PSILs was confirmed from the EDX data showing the presence of exchanged anions.

Swelling property of the resins was estimated in terms of the swelling volume of the PSILs in different solvents as summarized in **Suppl. Table 1**. Swelling property determines the diffusion tendency of the reactants on the polymer surface and hence, the overall kinetics and yield in the process. The polymer supported ILs normally swelled more in polar aprotic solvents rather than less polar organic solvents which can be attributed to the amphiphilic nature provided by the hydrophobic anion and hydrophilic –OH groups. Among the PSILs, PS-DESBA-NTf_2 exhibited more swelling in polar aprotic solvents like DMF, NMP and DCM than the others mainly due to the more hydrophobic nature of NTf_2^- than PF_6^-, BF_4^- or Br^-.

The synthetic efficiency of the PSILs in SPPS was investigated following the steps shown in **Scheme 2**. The dipeptide, Phenylalanyl leucine (Phe-Leu-OH) and pentapeptide, Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu), was synthesized using the same coupling-decoupling reaction sequences and characterized using ^{1}H NMR, MALDI-TOF and Circular Dichroism (CD) spectral data (**Suppl. Figure 5**).[9] Further, the yield and purity of the obtained peptide were estimated using HPLC data (**Suppl. Figure 3**) as shown in **Table 1**. All the PSILs showed higher yield and purity of the dipeptide (yield: 92-98%, purity 89-98%) and pentapeptides (yield: 82-95%, purity: 80-96%) than similar homogeneous phase IL supports (yield: 50-55%, purity: 86-90%).[5] Among the PSILs, PS-DESBA-NTf_2 was most efficient in terms of yield (95%) and purity of the pentapeptide (96%). Comparably lower efficiency of other PSILs composed of PF_6^-, BF_4^- or Br^- anions can be attributed to the inherent lower swelling property leading to less flexibility of the support matrix. The amphiphilic environment provided by the anionic component plays important role in the diffusion of the reagents over the resin surface. As more than one –OH groups were present in the side chain of the PSILs, almost two or three equivalent of amino acids could be loaded. Thus, higher loading efficiency was obtained using these PSILs than similar resin supports.[7,10] Further, in addition to the swelling property, the electrostatic environment provided by these ILs stabilizes the growing peptide chain and the reagents resulting in higher yield and purity of the obtained peptides.

To demonstrate the electrostatic effects of these ILs, the coupling kinetics of the first amino acid, Fmoc-Leu-OH, on the PSILs as well as the second amino acid, Fmoc-Phe-OH, on the Leu-OH-attached PSILs was investigated and the results are shown in **Figure 1**. The progress of the reaction was measured by collecting resin samples at different time intervals followed by Fmoc-titration as reported in literature.[11] All these PSILs showed high loading capacity as well as fast reaction rates proportional to their swelling properties. PS-DESBA-NTf_2 showed the maximum



Scheme 2. General outline of the peptide synthesis steps using PSILs.

Table 1. Yield and purity of the peptides produced via **Scheme 2** using different PSILs.[a,b]

Resins	Phe-Leu-OH[c]		Leu-enkephalin[d]	
	Yield (%)	Purity (%)	Yield (%)	Purity (%)
PS-Cl	79	82	45	68
PS-DESBA-Br	93	89	82	80
PS-DESBA-PF_6	95	96	89	95
PS-DESBA-NTf_2	98	98	95	96
PS-DESBA-BF_4	92	93	82	91
PS-TEA-NTf_2[e]	97	95	94	94

[a] Reaction conditions (coupling step): Temperature, 27°C; Resin, 0.15 g; Fmoc-amino acid, 1.2 mmol, HBTU, 1.2 mmol; DIPEA, 2.4 mmol; 5 mL DMF.
[b] Reaction conditions (Fmoc deblocking step): Temperature, 27°C; 20% (v/v) piperidine in 5 mL DMF.
[c] Reaction conditions (detachment step): Temperature, 27°C, 95% TFA, 5 mL.
[d] Reaction conditions (detachment step): Temperature, 27°C, TFA/triisopropysilane/H_2O (95:2.5:2.5), 5 mL.
[e] Fmoc-amino acid, 1.8 mmol.

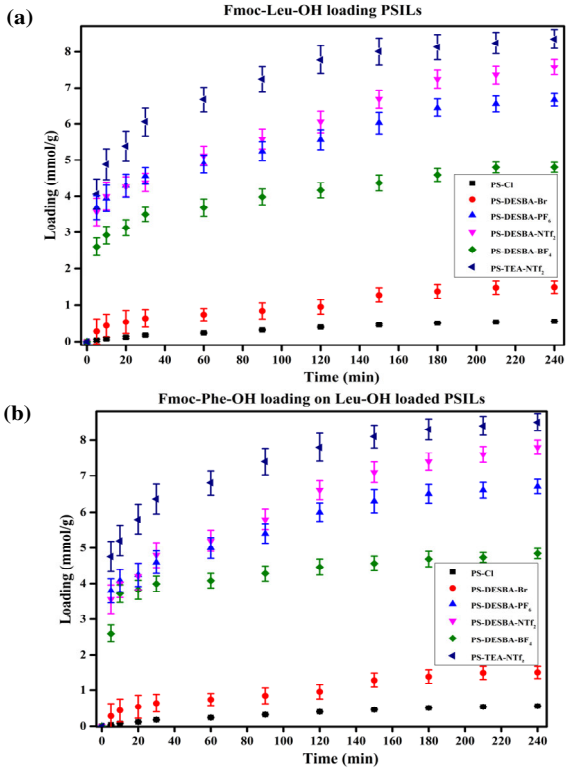


Figure 1. Kinetics of (a) Fmoc-Leu-OH loading on PSILs and (b) Fmoc-Phe-OH on Leu-OH loaded PSILs; Reaction conditions: Temperature, 27°C; Resin, 0.15 g; Fmoc-amino acid, 1.2 mmol, HBTU, 1.2 mmol; DIPEA, 2.4 mmol; 5 mL DMF.

reaction rate and higher loading efficiency. PS-TEA-NTf_2 showed higher loading efficiency as it contained three –OH functionalities. However, the loading efficiency of the PS-TEA-NTf_2 was not proportional to the number of –OH groups in contrary to the PSILs containing a 2-butyl group as a spacer to decrease the inter-chain interactions in between the two growing peptide chains. The growth of the three simultaneous peptide chains on the PS-TEA-NTf_2 support results in increased inter-chain interaction between the amino acids as evident from **Figure 1**. Further, this faster kinetics and efficient loading can be attributed to the flexible nature of these functionalized supports with –OH groups on the side chains and hydrophobic anions resulting in efficient biphasic reaction media.

In conclusion, a series of multifunctional ILs with different anionic entities were supported on PS resin and used in the manufacture of di- and pentapeptides. The modification of the resin supports with ILs containing –OH groups on the side chain and hydrophobic anions played important role in the swelling property of the polymer support. The kinetics of the first and second amino acid attachment on the polymer support was investigated and a model di- and pentapeptide was synthesized using these PSILs to demonstrate the synthetic efficiency. The inherent electrostatic interactions of the ILs was found to play crucial role in controlling the conformation of the growing peptide chain resulting in improved overall yield and purity of the peptides. Overall, a new class of amine based ionic liquid modified resin support is provided as a solution with new insights to get rid of the persisting problems in peptide manufacture processes.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/.

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Graphical Abstract

Multifunctional ionic liquid-bound polystyrene resin with high loading capacity as support in solid-phase peptide synthesis

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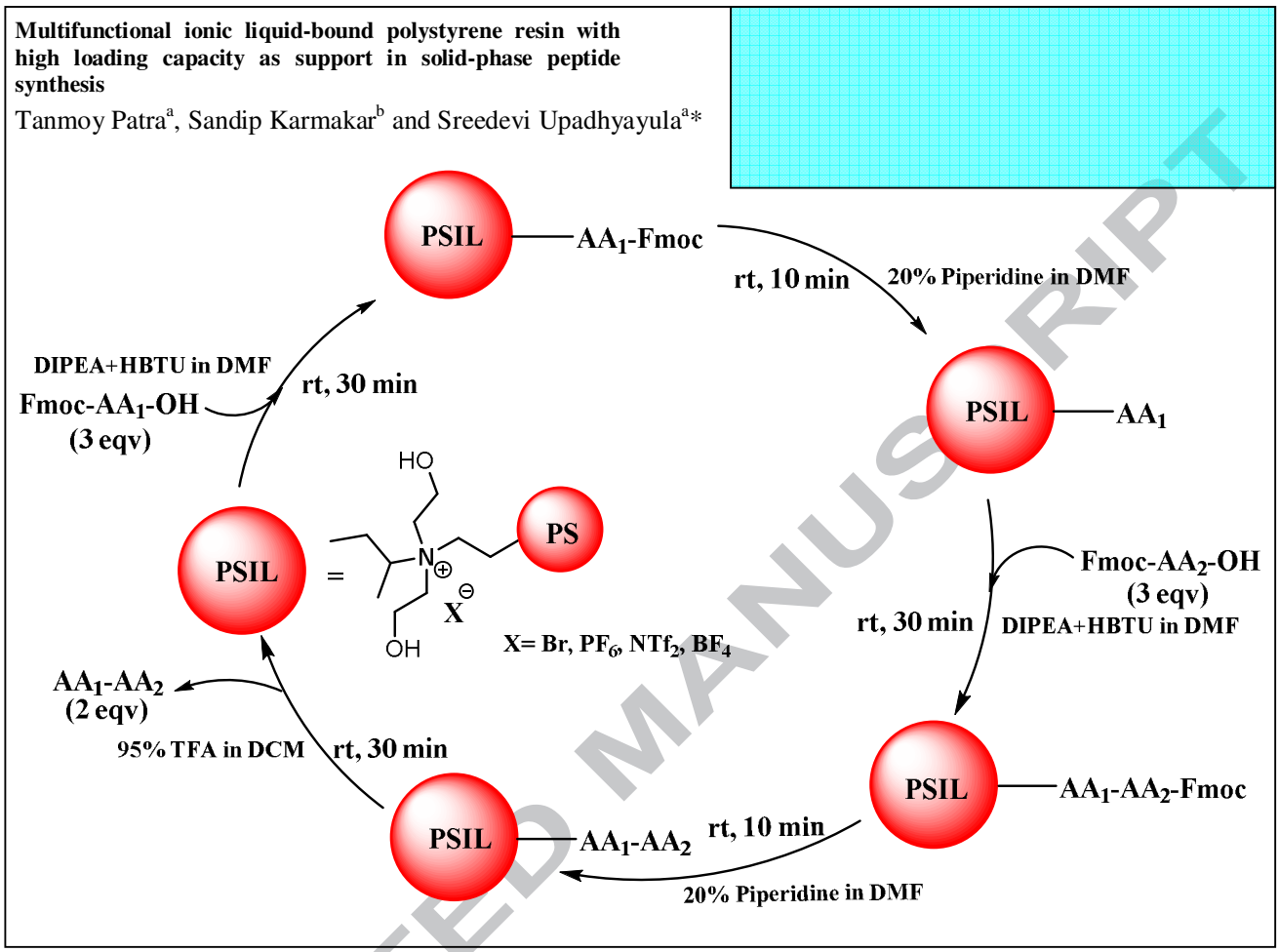

Highlights:

- Ionic liquid incorporated polystyrene resin (PSIL) in peptide synthesis
- Improved flexibility and swelling property of the resin
- High yield and purity of the di- and pentapeptides
- Kinetics of first and second amino acid loading on PSILs
- Controlled chain growth on PSILs with the stabilization of growing peptide chain