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New polyoxyalkyleneamine-grafted paramagnetic supports for solid-phase synthesis and bioapplications

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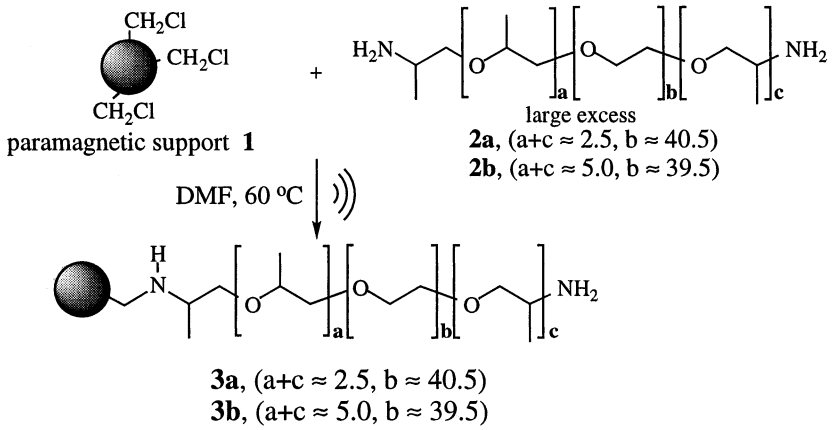
Abstract—The synthesis of some novel polyoxyalkyleneamine grafted paramagnetic supports for use in chemical and biological applications is described. These composite supports were synthesized by utilizing power ultrasound to enhance the level of incorporation of certain high molecular weight, polyoxyalkyleneamine (Jeffamine®) polymers into the matrix of a chloromethylated paramagnetic support. These new composite paramagnetic supports show excellent expansion properties in both organic solvents and water. The use of these grafted supports for multi-step solid-phase synthesis was then demonstrated by example.

Recently, we introduced a paramagnetic support for solid-phase synthesis and solution scavenging.[1] Instead of filtration, a magnet is used to separate the support from the soluble components of the reaction mixture. It is well known that incorporation of polyethylene glycol (PEG) within the matrix of 1–2% cross-linked polystyrene can improve the reaction kinetics of many solid-phase reactions as well as make the support compatible with aqueous solvents.[2–5] In fact, it has been shown that the bound molecules need not be directly attached to the ends of the grafted PEG in order to receive the benefits of the improved reaction kinetics.[6,7] We were interested in finding a simple and inexpensive way of grafting large molecular weight PEGs to our newly developed paramagnetic support **1** (Scheme 1). In this way, we would not only improve the solid-phase reaction kinetics of our paramagnetic support for solid-phase synthesis, but also be able to biologically screen the resulting synthesized compounds while they are still attached to the support.[8] The simplest route is to treat our chloromethylated paramagnetic support with a commercially available dihydroxy- or diamino-containing PEG under S_N2 reaction conditions. This route has been shown to be successful with low molecular weight PEGs (<700 mw), but problematic with PEGs of higher molecular weight ($\approx$2000 mw).[9–11] The main problem is



Scheme 1.

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Table 1. Comparison of swelling properties between grafted (**3a**,**b**) and ungrafted (**1**) supports[a]

Support	Solvents[b]	% Change in volume
1	DMF	56
3a,**b**	DMF	64
1	H_2O	0
3a,**b**	H_2O	33

[a] Based on 100 mg of resin. Average of three measurements.
[b] %ΔVolume = 100×((solvated volume−dry volume)/dry volume).

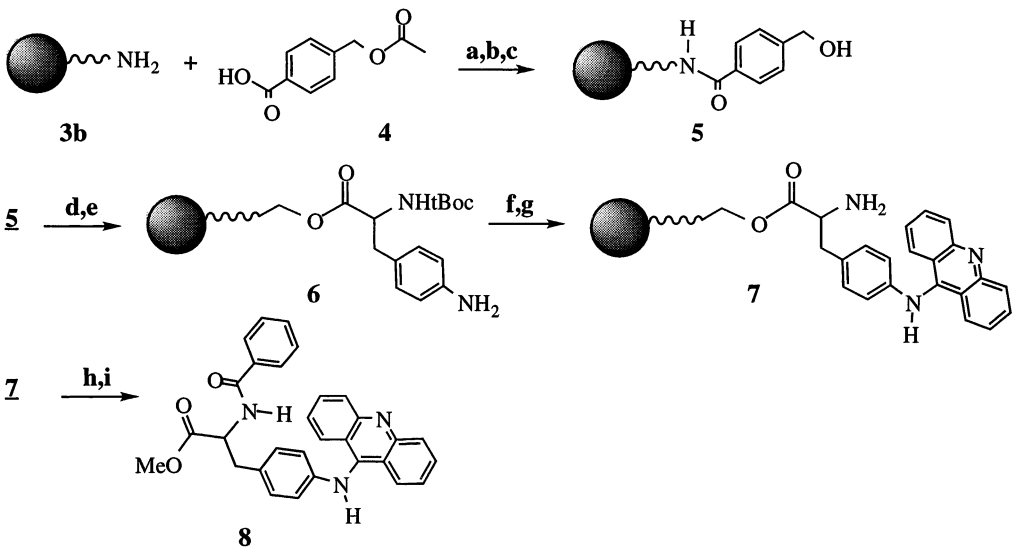
the difficulty in diffusing the higher molecular weight PEGs into the matrix of the cross-linked polystyrene for reaction to take place.

We have recently shown that power ultrasound can enhance the rate of selective solid-phase reactions by decreasing the time for solute molecules located within the inner and outer surfaces of a support to reach equilibrium.[12,13] We were interested in determining if power ultrasound could also be used to facilitate the grafting of these higher molecular weight PEGs by enhancing their diffusion rate into our chloromethylated paramagnetic support.

Chloromethylated paramagnetic support **1** (2–15 g, 1.0 mmol chlorine/g) was treated with an excess (6 equivalents) of either of the polyoxyalkyleneamines (Jeffamine®) **2a** or **2b** in dimethylformamide (DMF) at 60°C in the presence of power ultrasound (50% power) for the first 8 hours and then heated without ultrasound (no agitation) for an additional 10 hours (Scheme 1).[13,14] A magnetic separator composed of a series of embedded neodymium permanent magnets was then used to separate the paramagnetic support from the milky white reaction mixture which was then removed by aspiration. Each support was then washed twice with DMF, eight times with water, three times with methanol and twice with methylene chloride in that order. Each wash consisted of adding the appropriate solvent to the support and then sonicating the mixture under low power for 3–5 minutes, magnetically separating the support and then aspirating off the solvent. The supports were then placed in a soxhlet extractor and washed with hot methanol for 3–4 hours. The supports were then dried under reduced pressure to give the Jeffamine® grafted supports **3a** and **3b**, which exhibited an average polyoxyalkyleneamine loading capacity of 0.23 mmol Jeffamine®/g (±0.02 mmol/g) for each of the two supports.[15,16] This is in contrast to when power ultrasound is not used in which case one observes a 30–40% drop in the overall Jeffamine® loading level.[17]

Both Jeffamine® grafted supports (**3a**,**b**) exhibited identical swelling characteristics in both organic and aqueous solvents. Additionally, both supports were found to have slightly greater swelling characteristics in dimethylformamide than that found with the ungrafted support **1** (Table 1).

Because of their superior swelling characteristics paramagnetic supports **3a**,**b** can be used for both chemical and biological applications. Here we present an example demonstrating the robustness of this type of support for multi-step solid-phase organic synthesis. To the Jeffamine® grafted paramagnetic support **3b** (0.42 mmol amine) was coupled the acetate-protected hydroxymethylbenzoic acid linker (HMBA) **4** (Scheme 2). The support was then treated with an excess of acetic anhydride, diisopropylethylamine (DIEA) in DMF to cap any unreacted amines. The linker was deprotected with hydrazine hydrate to give the resulting free alcohol **5**. Doubly protected 4-aminophenylalanine was then coupled using DIC, dimethylaminopyridine (DMAP) and DIEA in DMF. The Fmoc protecting



Scheme 2. *Reagents*: (a) DIC, DMAP, DMF; (b) acetic anhydride, DIEA, DMF–CH_2Cl_2 (capping step); (c) 1 equiv. hydrazine hydrate, DMF; (d) 10 equiv. Boc-Phe(4-NHFmoc)-OH, 5 equiv. DIC, 2 equiv. DMAP, DMF; (e) 50% piperidine–DMF; (f) 20 equiv. 9-chloroacridine, 20 equiv. DIEA, DMF, 60°C 10 h; (g) 35% TFA–CH_2Cl_2; (h) 10 equiv. benzoyl chloride, 10 equiv. DIEA, DMF; (i) NaOMe–MeOH–THF.

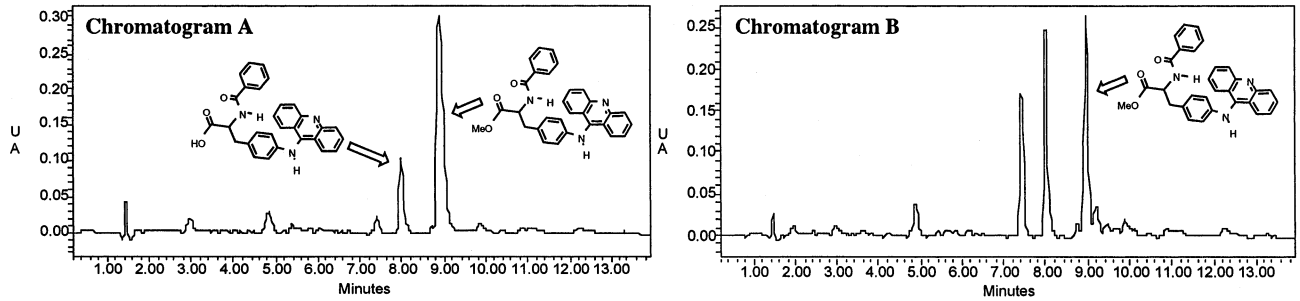


Figure 1. (A) Reversed-phase HPLC chromatogram monitored at 220 nm of crude cleaved product **8** synthesized on grafted support **3b**. (B) Reversed-phase HPLC chromatogram monitored at 220 nm of crude cleaved product **8** synthesized from a non-grafted aminomethylated version of support **1**.

group was then removed to give the resulting free amine **6** (0.14 mmol amine/g). Free amine **6** was then treated with a large excess of 9-chloroacridine and DIEA in DMF at 60°C to give the resulting resin-bound 9-amino acridine product which was then treated with 35% TFA–methylene chloride to give the resulting free amine **7** (Scheme 2). The resin bound free amine was then treated with an excess of benzoyl chloride and DIEA in DMF followed by treatment with sodium methoxide in methanol–THF to give the cleaved phenylalanine–acridine product **8**, as the methyl ester, in 62% overall yield starting from resin bound amine **6**.[18]

The reversed-phase HPLC chromatogram of the crude product of **8** can be seen in Fig. 1A. As a comparison of the effectiveness of utilizing the Jeffamine® graft during the synthesis, compound **8** was simultaneously synthesized using a non-grafted aminomethylated version of paramagnetic support **1**.[1]

Comparison of the two chromatograms shows a marked reduction in the number of impurities from the Jeffamine® grafted support **3b** (Fig. 1A) as compared to the non-grafted support (Fig. 1B).

In conclusion, polyoxyalkyleneamine functionalized paramagnetic supports have been synthesized utilizing high powered ultrasonic mixing to facilitate a high level of Jeffamine® incorporation within the matrix of the support. These new paramagnetic supports have been shown to be stable during multi-step solid-phase synthesis while exhibiting excellent solvent expanding properties in water. In addition, preliminary evidence suggests that these supports have improved reactivity for solid-phase synthesis, which would be an aid in their use as a platform for the synthesis and biological screening of compounds within single beads, an area of current interest in our laboratory

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16. Average of two runs for each support. Loading capacity was determined by both nitrogen elemental analysis (Eq. (1)) and by measuring the weight change of the resin after the reaction (Eq. (2)). Both measurements were found to

be in agreement to within 5% of each other. Elemental analysis showed less than 0.05% chlorine remaining in supports **3a** and **3b**.

$$\text{Primary amine substitution (mmol/g)} = (\%\text{nitrogen} \times 10)/(2 \times MW_{\text{nitrogen}}) \quad (1)$$

$$\text{Jeffamine}^{\circledR}\ \text{substitution (mmol/g)} = (1000 \times \Delta W_{\text{resin}})/(\Delta MW_{\text{group}} \times W_{\text{resin}}),\ \text{where } \Delta MW_{\text{group}} = \text{difference in MW of added group; } \Delta W_{\text{resin}} = \text{weight change of resin.} \quad (2)$$

17. The reaction was stirred at 60°C for 24 h following the same work up procedure.
18. **8**: ^{1}H NMR (CD_3OD) δ 7.99–7.94 (m, 3H), 7.78–7.61 (m, 6H), 7.43 (t, 1H, $J=7.9$ Hz), 7.26 (d, 1H, $J=8.4$ Hz), 7.22–7.18 (m, 4H), 7.08 (t, 1H, $J=7.9$ Hz), 6.95 (d, 3H, $J=8.4$ Hz), 4.77 (dd, 2H, $J=5$ Hz, $J=5$ Hz), 3.77 (s, 3H), 3.0 (dd, 1H, $J=10.5$ Hz, $J=10.5$ Hz); ^{13}C NMR (CD_3OD) δ 172.0, 162.3, 133.1, 132.9, 131.4, 129.5, 128.4, 127.0, 123.7, 122.8, 121.6, 121.5, 119.0, 55.5, 53.0, 37.4; FABMS (M+H) calcd for $C_{30}H_{25}N_3O_3$ m/e 476.1974, measured 476.1963.