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New High Loading Paramagnetic Support For Solid Phase Organic Chemistry

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Abstract: A paramagnetic support exhibiting very high loading capacities is described. The use of this novel support in solid phase organic synthesis and affinity chromatography is presented.

Solid-supported organic synthesis has been of great utility in assisting the production of combinatorial libraries for biological screening.[1] Many of the supports used in solid phase organic synthesis have also been utilized for purifying combinatorial libraries produced in solution.[2-4] The major advantage in producing chemical libraries by solid-supported synthesis over traditional solution synthesis is the relative ease in separating starting materials from product. This rapid ability to separate starting materials from product can also facilitate the library's production through automation. In most solid phase organic synthesis reactions the physical separation of the support from the solubilized components of the reaction mixture has primarily been accomplished by filtration through a glass or polymer filter. Although filtration has been the method of choice, it does have limitations that warrant the development of new approaches. One such limitation is the difficulty in automating the simultaneous washing and filtration of hundreds of small scale solid-phase reactions.

A field that has had some success in automating some of its techniques is immunodiagnostics. Exposing antibody-bound paramagnetic beads to a magnetic field can be used to separate antibody-bound antigen from unbound antigen in immunoassays.[5] The use of magnetic separation in the field of solid-supported organic chemistry has been slow in coming due to the instability and/or relatively poor loading capacities exhibited by the currently available paramagnetic supports (typically < 0.2 mmole reactive groups/gram).[6-7] One way to make paramagnetic polymer beads stable in organic solvent is to enhance the levels of cross-linking around the magnetite core. A major problem with this approach is that higher levels of cross-linking (>1-2%) reduce the extent to which the support expands and contracts and also decrease the level of reactivity.[8] A solution to this problem involved synthesizing a composite paramagnetic bead incorporating zones of high cross-linking in only those areas where the magnetite is located. The simplest way of creating such a composite was to first create primary beads **1** (5-10 micrometers in diameter) composed of magnetite crystals encased in high cross-linked polystyrene, then to enmesh these primary beads **1** into a larger bead composed of 1-2% cross-linked polystyrene (100-200 micrometers in diamter).[9,10] In order to incorporate functionality into the new composite bead, a functionalized styrene monomer such as chloromethyl styrene was used to enmesh the primary beads **1** to give the resulting chloromethylated, polystyrene composite bead **2** (1 mmole chloromethyl groups/gram of

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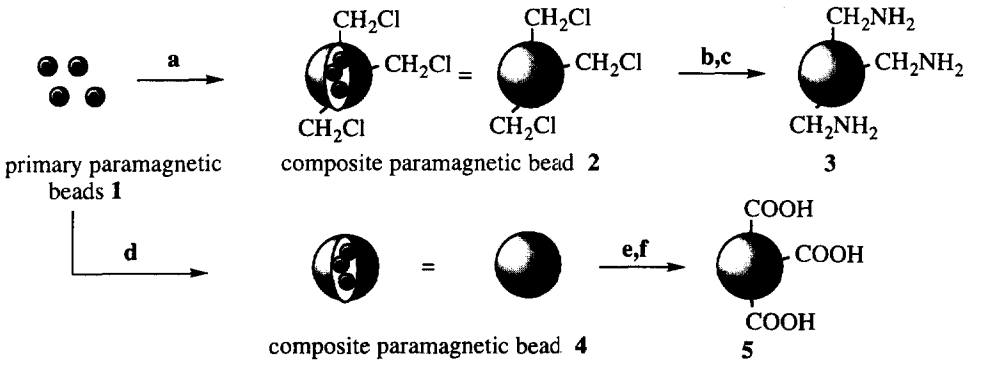
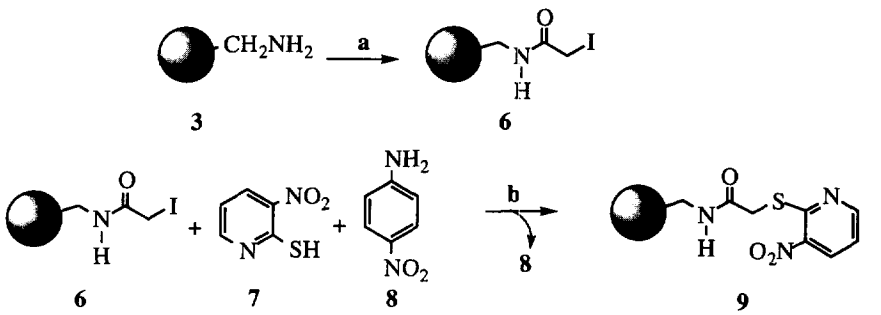
paramagnetic bead). Treating the chloromethylated support **2** with potassium phthalimide in refluxing dimethylformamide followed by deprotection with hydrazine gave the resulting aminomethylated composite support **3** (0.7-0.9 mmole aminomethyl groups/gram). Alternatively, the primary beads can be enmeshed with unfunctionalized polystyrene and subsequently functionalized by treating the support with butyllithium and an electrophile such as CO_2 to give the resulting carboxyl functionalized support **5** (0.5 mmole carboxy groups/gram, Scheme 1). The composite beads **2-5** are stable and expand in organic solvents and exhibit loading capacities that are comparable to those of standard solid phase synthesis supports.[9-10]



Scheme 1 Reagents: (a) chloromethylstyrene; (b) potassium phthalimide; (c) N_2H_4, ethanol; (d) styrene (e) butyllithium, CO_2; (f) H^+.

These functionalized, high loading composite supports **2,3 & 5** can be used in a variety of different organic chemistry applications. For example, treating the aminomethylated composite support (**3**) with iodoacetic acid and diisopropylcarbodiimide (DIC) produces an iodoacetamide functionalized support **6** (0.64 mmole iodoacetamides/gram of support, Scheme 2).[11] This derivatized support can be used as a high loading scavenger resin for free thiols in solution.

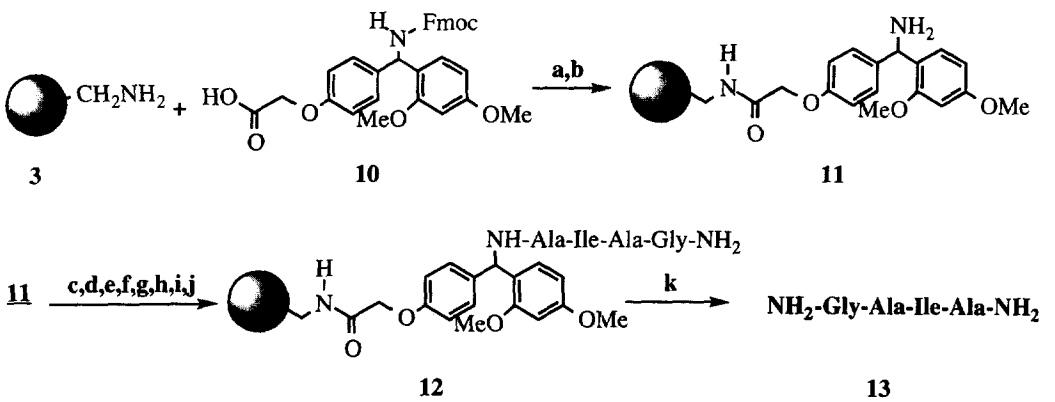


Scheme 2 Reagents: (a) iodoacetic acid, DIC, methylene chloride; (b) DIEA, DMF.

As a demonstration of this application, iodoacetamide support **6** (300 mg, 0.19 mole of iodoacetamide) was

added to a mixture composed of equal molar quantities (0.06 mmole) of 2-mercapto-3-nitropyridine (**7**) and 4-nitroaniline (**8**) and diisopropylethylamine (DIEA) in dimethylformamide (DMF) as solvent. After stirring the mixture for 26 minutes, 98% of the mercaptan (**7**) was found to bind to the resin.[12] The particles were then separated using a magnet and the solvent removed by aspiration. The resin was then washed with DMF, methanol and methylene chloride and then dried under reduced pressure. Elemental analysis of the dried resin **9** showed a sulfur substitution level of 0.48 mmole of sulfur/gram of resin.

These high loading composite supports can also be used in solid phase organic synthesis. For example, treating 0.5 gram (0.7 mmole of aminomethyl groups/gram) of the aminomethylated composite support (**3**) with an excess of the [p-(R,S)-a-[1-9H-fluoren-9-yl)-methoxybenzyl]-phenoxyacetic acid linker and DIC in DMF gave the resulting linker bound paramagnetic support which upon treatment with 50% piperidine in DMF gave the resulting resin bound free amine (**11**).[13] The resin was separated using a magnet and the soluble components of the reaction removed by aspiration. The resin was washed with DMF, methanol and methylene chloride in that order. After each wash the resin was magnetically separated and the liquid aspirated off. Following a standard peptide synthesis protocol consisting of an excess of fluorenylmethoxycarbonyl (Fmoc) protected amino acid, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium-hexafluorophosphate (PyBop) coupling agent and DIEA in DMF the resin bound tetrapeptide (**12**) was produced (Scheme 3). Cleavage of the peptide was accomplished by successive treatments of the resin with 10% trifluoroacetic acid (TFA) in methylene chloride. After each acid treatment, the resin was separated magnetically and the solvent siphoned off. The combined acid washes were then evaporated under reduced pressure and the resulting oil precipitated from ether to give the resulting GAIA tetrapeptide (**13**) as the TFA salt (30% overall yield).[14]



Scheme 3 Reagents: (a) DIC, DMF; (b) 50% piperidine-DMF (deprotection step); (c) Fmoc-Ala-OH, PyBoP, DIEA, DMF; (d) deprotection; (e) Fmoc-Ile-OH, PyBoP, DIEA, DMF; (f) deprotection; (g)Fmoc-Ala-OH, PyBop, DIEA, DMF; (h) deprotection; (i) Fmoc-Gly-OH, PyBop, DIEA, DMF; (j) deprotection; (k) 10% TFA-methylene chloride.

In conclusion, a series of functionalized, high loading, paramagnetic supports have been developed which exhibit loading capacities that not only surpass any previously published paramagnetic support but also are comparable to standard solid phase synthesis supports used today. These new supports, which are stable in organic solvents, can be used both in practical solid phase organic synthesis and as scavenging resins for the purification of combinatorial libraries made in solution.

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References and Notes

1. For a recent review see: Hall, S.E. in *Annual Report in Combinatorial Chemistry and Molecular Diversity, Vol. 1*, (Moos, W.H.; Pavia, M.R.; Kay,B.K.; Ellington, A.D., editors), Escom Sciences Publishers, Leiden, **1997**, 30-40.

2. Gayo, L.M.; Suto, M.J. *Tetrahedron Lett.* **1997**, *38*, 513.

3. Siegel, M.G.; Hahn, P.J.; Dressman, B.A.; Fritz, J.E.; Grunwell, J.R.; Kaldor, S.W. *Tetrahedron Lett.* **1997** *38*, 3357.

4. Kaldor, S.W.; Siegel, M.G.; Fritz, J.E.; Dressman, B.A.; Hahn, P.J. *Tetrahedron Lett.* **1996**, *37*, 7193.

5. Matte, C.; Muller, A. **1994**, US 5,318,914.

6. Szymonifka, M.J.; Chapman, K.T. *Tetrahedron Lett.* **1995**, *36*, 1597.

7. Benner, S.A. **1987**, US 4,638,032.

8. A low cross-linked polystyrene bead (1-2% divinyl benzene) can be seen as a sponge with the majority (>95%) of the reactive sites located within the cavities of the bead. Solvents such as methylene chloride cause the cavities within the bead to greatly expand, allowing reagents and starting materials to freely enter and leave the bead through the process of diffusion.

9. Sucholeiki, I.; Margetts, G.; Roberts, M. **1998**, US 5,834,121.

10. Sucholeiki, I., **1997**, US 5,684,130.

11. The level of substitution was determined by iodine elemental analysis.

12. The reaction was followed by taking aliquots at timed intervals and separating the components using reversed-phase HPLC (C-18 radial compression column) and 254 nm detection.

13. The loading capacity was determined to be 0.6 mmoles amine/gram by spectrophotometric assay of the released fluorenylmethoxycarbonyl (Fmoc) group according to Green, J.; Bradley, K. *Tetrahedron* **1993**,*49*, 4141.

14. Tetrapeptide **13**: ^{1}H NMR (d-DMSO) δ 0.83-0.85 (m, 6H), 1.08-1.1 (m, 1H), 1.22 (t, 6H, J=7.8 Hz), 1.40-1.44 (m, 1H), 1.70-1.77 (m, 1H), 3.57 (br s, 2H), 4.12-4.21 (m, 2H), 4.40-4.50 (m, 1H), 6.98 (s, 1H), 7.28 (s, 1H), 7.75 (d, 1H, J=7.1 Hz), 7.96-8.05 (m, 4H), 8.50 (d, 1H, J=7.1Hz); amino acid analysis Gly(1.0), Ala(2.2), Ile(1.1); FABMS (M+H) Calcd for $C_{14}H_{27}N_5O_4$ m/e 330.2141, measured 330.2141. Anal. Calcd for $C_{14}H_{27}N_5O_4 \cdot 1.35$ TFA: C, 41.50; H, 5.91; N, 14.48; F, 15.91. Found: C, 41.17; H, 5.80; N, 12.98; F, 15.87.