# Solid-Phase Dendrimer Synthesis and the Generation of Super-High-Loading Resin Beads for Combinatorial Chemistry

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Dendrimers have been attracting much attention of late.[1] As structurally fascinating molecules they do much to spur the imagination of the organic chemist. Aesthetics aside, these new materials have been suggested to have promise in a whole host of important potential applications, ranging from specialized drug-delivery systems to catalyst carriers. Two main methods exist for the synthesis of dendrimers: a divergent approach, where the dendrimer is assembled in a totally linear manner,[2] or a convergent method where fragments of the dendrimer are condensed together.[3] These two methods both suffer from major problems when it comes to practical synthesis, in particular, the necessity for repeated and time-consuming purifications. The solid-phase synthesis of dendrimers would have many advantages. Firstly, large excesses of reagents could be used without the problems usually associated with purification, which becomes only a matter of extensive washing. Secondly, the use of differentially protected starter units would allow an avenue into the synthesis of unsymmetrical dendrimers under very clearly defined reaction conditions and allow the synthesized dendrimer to be specifically functionalized to other molecules of choice. This would be especially practical using the orthogonally protected polyamine linkers we have previously reported[4] and could be particularly important following a very recent paper detailing the use of dendrimers for solution-based combinatorial applications.[1d] We also anticipated that the dendrimers generated using solid-phase techniques would be much more homogeneous than those prepared using conventional solution methodology. At the present time, there is a huge demand for solid-phase synthetic resins,[5] in particular, aqueous compatible based resins.[6] These do, however, suffer from relatively low loadings and high cost. It appeared to us that if resin-bound dendrimers could be made we would have the ability to enhance resin loading by at least 1 order of magnitude. Resin-bound dendrimers made from lysine[7] are commercially available for multiple-antigenic peptide synthesis but only with relatively low loadings, at some expense, and on solid supports incompatible for the solid-phase synthesis of small organic molecules.

We therefore synthesized two polyamidoamine (PAMAM) dendrimers on the solid phase starting from TentaGel resin-bound linker **1**.[4] This acid-labile linker allows cleavage of the dendrimer from the resin when required for analytical control of dendrimer synthesis. Polystyrene resins proved unsuitable for PAMAM dendrimer synthesis, whereas the polystyrene−poly(ethylene glycol) resins (PS−PEG e.g., TentaGel) worked well.[8] The only problem with these resins was the cleavage of variable amounts of PEG under the acidic cleavage conditions as evidenced by NMR ($^1$H and $^{13}$C). Dendrimer synthesis started from the initiator core-linker conjugate **1**, which was treated with 250 equiv of methyl acrylate in MeOH for 12 h followed by removal of excess reagents by filtration and extensive washing (MeOH, $Et_2O$) before being treated with 250 equiv of 1,*n*-diaminoalkane ($n$ = 2, 3) for 24 h (these reagents were typically recycled). To monitor the consumption and generation of resin-bound primary amines, we used the sensitive ninhydrin assay in both a quantitative and qualitative manner,[9] as well as our recently reported method[10] for *in situ* cleavage and the monitoring of solid-phase reactions and the cleavage of material at each half generation from 10−100 beads and ES-MS analysis. These methods allowed dendrimer synthesis to be controlled by analyzing for the presence of dendrimer defects and incomplete reaction.

Samples of the 0.5 and 1.0 generation dendrimers (**3**, **5a**, and **5b**) were cleaved initially using 100% TFA, with the resin previously swollen with $CH_2Cl_2$. The dendrimers themselves were essentially homogeneous although contaminated with PEG. Milder cleavage conditions (50% TFA in $CH_2Cl_2$) were more successful in reducing the level of cleaved PEG, but complete elimination of this problem will require the use of alternative PEG-based resins. Dendrimer synthesis was continued as above by repeated treatments of the resin with methyl acrylate and diamine (Scheme 1). Dendrimer generation 3.0 (**13b**) when cleaved and analyzed by $^1$H and $^{13}$C NMR indicated the material was relatively homogeneous; however, ES-MS showed the presence of a defect, a portion of the material with both ends of a diamine having displaced methyl esters. However, even this defect will have 14 amine groups available for chemistry arising from a single starting amine functionality and thus still contributes to the resin-loading enhancement. This generation 3.0 dendrimer TentaGel resin had a substitution of approximately 2.3 mmol $g^{-1}$, with each bead having between 5 and 6 nmol of free amino groups. This material was converted to a generation 4.0 dendrimer (a total of 32 amine groups now originating from a single resin amino function) and a resin loading of theoretically 2.8 mmol $g^{-1}$; or a single bead having approximately 9.6 nmol bead$^{-1}$. The loading per bead naturally doubles during this process, although the substitution only

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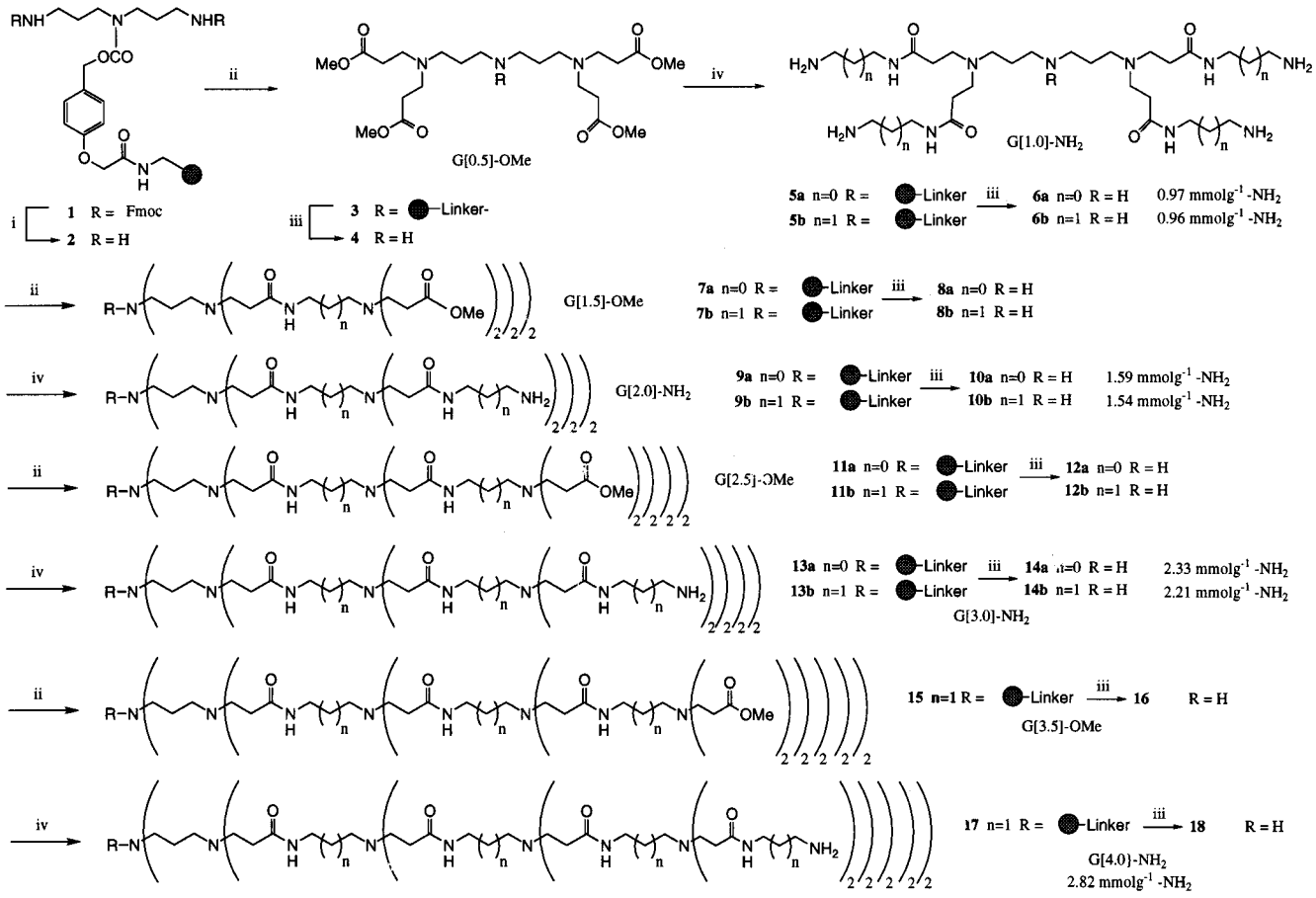
**Scheme 1. Solid-Phase Synthesis of PAMAM Dendrimers**[a]




[a] (i) 20% piperidine/DMF; (ii) $CH_2{=}CHCO_2Me$, MeOH; (iii) TFA; (iv) $H_2NCH_2(CH_2)_nCH_2NH_2$, MeOH.

increases from 2.3 to 2.8 mmol $g^{-1}$ due to the increased mass of the dendrimer core.

The generation 3.0 dendrimer-linked TentaGel (**13b**) was used to synthesize a dipeptide using the super acid-sensitive linker 4-[4-(hydroxymethyl)-3-methoxyphenoxy]-butyric acid (HMPB) and Fmoc chemistry. This dual linker approach allowed selective cleavage of the peptide from the dendrimer using 1% TFA in $CH_2Cl_2$ without cleaving the dendrimer from the resin.[11] The dipeptide was produced in an overall yield of 44% from the initial TentaGel resin (13 steps, average yield 94%) and was >95% pure as determined by HPLC. Attachment of Fmoc-Lys($^t$Boc)-Gly to the dendrimer (**13b**) followed by treatment with 50% TFA in $CH_2Cl_2$ allowed the release of the dendrimer-bound peptide. This was characterized by HPLC and ES-MS and showed the success of the whole synthetic process. It should again be noted that the initial bead loading of 2.3 mmol $g^{-1}$ will decrease during the synthesis due to the substantial increase in mass of the compound attached to the resin; obviously, the loading per bead remains constant. To assess some of the physical properties of these new beads, the dendrimer generation 3.0 beads (**13b**) were swollen in water and dichloromethane and analyzed under a microscope; the average ($n = 25$) diameters were 0.267 mm and 0.280 mm, respectively. These diameters were compared to those of unmodified TentaGel beads in water (0.219 mm) and dichloromethane (0.265 mm), showing the water compatibility of these new beads.

The solid-phase generation of dendrimers is ideal as a quick and simple approach to many dendrimeric structures, and by use of unsymmetrical polyamine starting units offers a convenient method of producing tagged or unsymmetrical dendrimer structures. All of these dendrimers have potential as an efficient method for enhancing resin loading in a very fast and efficient process. At a minimum, they provide a very high loading resin, which is water compatible and should provide the basis for a number of solid-phase applications. As noted above, going from dendrimer generation 3.0 to 4.0 only increases resin substitution from 2.3 to 2.8 mmol $g^{-1}$ due to the increased mass of the dendrimer core. For these reasons and because of the accumulating number of defects expected at higher generations, we believe that the generation 3.0 dendrimer would appear optimal for synthetic applications. An added advantage of generation 3.0 over 4.0 is the reduced likelihood of interstrand side reactions as well as reduced steric encumbrance.

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**Supporting Information Available:** Synthetic procedures and analytical data (10 pages).

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