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High-loading resin beads for solid phase synthesis using triple branching symmetrical dendrimers

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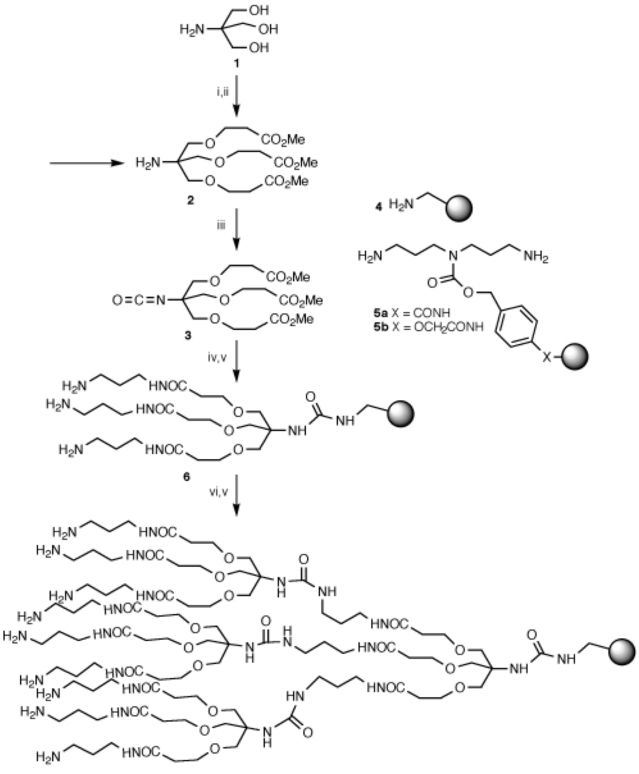
Resin beads with a loading of 200 nmol per bead have been prepared using a Generation 2.0 tris-based dendrimer to amplify resin loading sites.

The process of split and mix by its very nature rapidly generates large libraries of compounds in such a manner that a single resin bead carries a single compound. It is one of the most efficient and cost effective chemical methods for the generation of large compound numbers,[1] with compound numbers increasing exponentially with only a linear increase in the number of synthetic steps. The single bead/single compound concept opens up the option of screening compounds released from single beads as a powerful tool for high throughput screening and thus several major pharmaceutical companies now use single bead screening in anger in the area of lead generation. The drawbacks to this process are the tiny amounts of compound found on single beads, usually in the order of a few hundred picomoles, and the identification of the compound on the bead. Compound quantity maybe sufficient for one or two biological assays but it is usually insufficient for IC$_{50}$ determinations and chemical analysis/determination of the structure of the compound. These analysis issues have led to a number of elegant tagging solutions that encode the chemical sequence to which a bead has been exposed, although complicating the synthetic process.[2] However the need for encoding and resolution of the screening problems could be overcome if the loading of the solid support could be increased to a more practical level. We previously reported the synthesis of polyamine based Generation 3.0 PAMAM dendrimers on resins beads as a means of enhancing loading.[3]

Here we describe a practical approach to the synthesis of a tri-branching symmetrical dendrimer on the solid phase with an 18-fold amplification of resin loading per bead. Beads with a loading of 200 nmol per bead were prepared and used in a number of synthetic applications, with single beads containing more than sufficient compound for conventional analysis, including NMR!

The trifunctional dendrimer monomers[4] were prepared in bulk (> 50 g) by alkylation of tris **1** with acrylonitrile followed by nitrile hydrolysis in a saturated solution of HCl in dry MeOH to give the methyl ester **2**. Alternatively, the triacid could be obtained by direct hydrolysis. The hindered amine **2** was converted to the isocyanate **3** by treatment with Boc$_2$O and DMAP as described by Knölker[5] to give the stable symmetrical monomer **3**. Aminomethylpolystyrene resin (0.7 mmols g^{-1}, 250–300 μm, Polymer Laboratories) was directly derivatised with **3** as shown in Scheme 1. Displacement of the methyl ester by propane-1,3-diamine was followed by on-bead IR (ATR) and NMR analysis of cleaved material.† Repeating the process gave Generation 2.0 dendrimer beads with a loading of 85% of the theoretical maximum (36 nmol per bead). Swelling studies (Fig. 1) showed that the dendrimer beads swelled to a much greater degree in more polar solvents than the starting aminomethyl resin, as expected due to the nature of the dendrimer itself. It should however also be noted that swelling was measured in units of ml g^{-1}. Since the dendrimer beads have a density that is different to those of polystyrene (the mass of the generation 2.0 dendrimer is 2111 Da), trends rather than absolute swelling ratios in terms of bead sizes are being observed. The average diameters of the resin beads in DMF were: aminomethyl resin, 310 μm, PS-Gen 1.0, 400 μm and PS Gen. 2.0, 500 μm. These beads were extremely stable to solvation changes and extended manipulations and behaved in all respects as normal resin beads. They proved to be ideal for a broad range of chemistries. In addition, with *ca.* 27 000



Scheme 1 *Reagents and conditions*: i, acrylonitrile, 1,4-dioxane, 40% KOH, room temperature, 3 days; ii, HCl, MeOH, 4–6 h, reflux; iii, Boc$_2$O, DMAP (cat), CH$_2$Cl$_2$, room temp., 30 min; iv, **4** or **5a**,**b**, DMAP (cat), Pr$^i_2$NEt (2 equiv.), CH$_2$Cl$_2$ or DMF, 12 h; v, propane-1,3-diamine, MeOH, 3 days or propane-1,3-diamine, DMSO, 3 days; vi, **3**, DMAP (cat), Pr$^i_2$NEt (2 equiv.), DMF, 12 h.

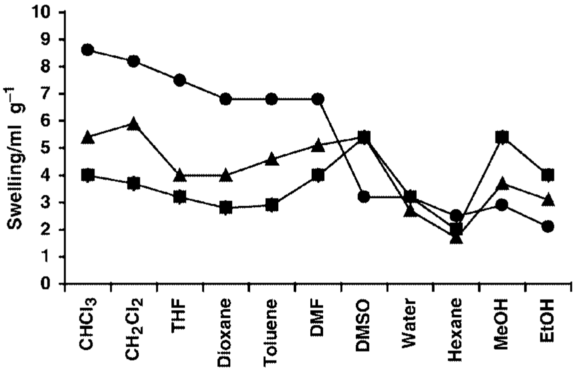


Fig. 1 Swelling studies: (●) aminomethyl resin; (▲) PS Gen 1.0; (■) PS Gen 2.0.

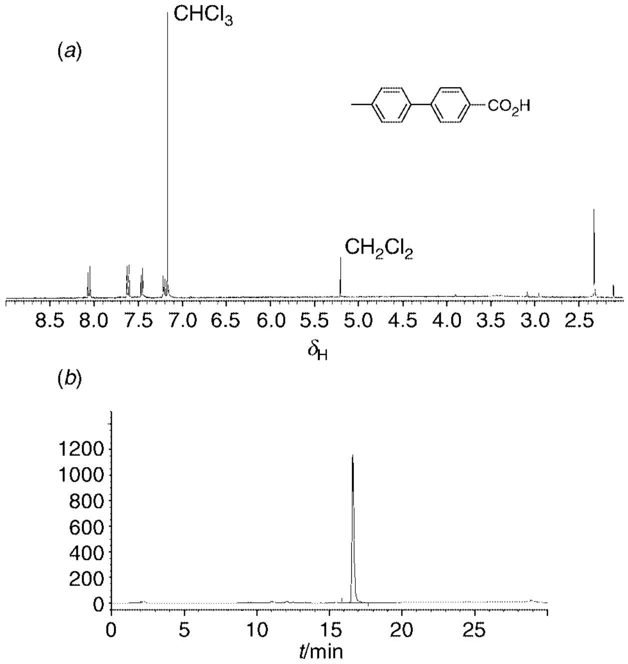


Fig. 2 (*a*) Crude ^{1}H NMR spectrum (5 mm NMR tube, 256 scans, 400 MHz) and (*b*) HPLC trace (10% of sample injected, 3.6 mm × 18 cm, C-18 column) for the Suzuki biaryl product cleaved from a single resin bead.

beads g^{-1} a split and mix synthesis on a gram of resin beads clearly provides the numbers of compounds and the amounts necessary for a serious single bead screening approach.

Dendrimer synthesis was also carried out on 400–500 μm aminomethyl polystyrene resin beads, loaded with the polyamine scaffolds **5a**,**b**,[3] as shown in Scheme 1. Generation 1.0 (8000 beads g^{-1}) had a loading of 116 nmol bead^{-1}, while Generation 2.0 (4300 beads g^{-1}) had a loading of 230 nmol bead^{-1}.‡ The dendrimer beads were used in a number of synthetic applications attaching a linker onto the free amino groups of the dendrimer to allow cleavage of the desired compound. This included peptide chemistry (synthesis of Fmoc-Val-Phe-Ala-OH) and a Suzuki coupling.[6] Thus 4-iodobenzoic acid was esterified onto the HMPB linker and 4-methylbenzene boronic acid coupled under standard conditions [K_2CO_3, $Pd(PPh_3)_4$, DMF, 110 °C, 16 h]. Cleavage of the biaryl compound from a single resin bead gave the HPLC and NMR data shown in Fig. 2. It should be noted that these very large beads were not as robust as the smaller beads used earlier, with solvent shock often causing the beads to shatter, necessitating a gradual change from one solvent to another and gentle handling, thereby limiting their utility.

We have thus devised a fast and efficient method of generating symmetrical tri-branched resin linked dendrimers in a very efficient manner. The smaller, robust Gen 2.0 beads with a loading of 36 nmol bead^{-1} (which has been extended to Gen 3.0, beads with a loading of 100 nmol bead^{-1}) are we believe ideally practical for single bead screening applications.

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Notes and references

† Using cleavable linkers dendrimers were cleaved at the 0.5*n* and *n* generations and fully analysed, and clearly showed that dendrimer synthesis had proceeded cleanly and as anticipated.

‡ By Fmoc determination.

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