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Rapid synthesis of high-loading resins using triple branched protected monomer for dendrimer synthesis

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Abstract—Resins with loading up to 96 nmol/bead were prepared by solid-phase dendrimerisation using a symmetrical 1→3 *C*-branched isocyanate monomer. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Since its first introduction by Merrifield in 1963,[1] solid-phase synthesis has evolved to become a key component in the drug discovery process within the pharmaceutical industry.[2] Although it was primarily developed for peptide and oligonucleotide synthesis, solid-phase synthesis of small organic molecules has recently undergone a huge expansion with many compounds of therapeutic interest having been synthesised on the solid-phase.[3] This can be explained by the increasing number of organic reactions which have been adapted for solid-phase chemistry,[2,4] together with the wider range of linkers developed for synthesis and cleavage of library compounds.[5] A variety of solid supports have also been developed to accommodate the broad range of chemical transformations necessary for library synthesis as well as a number of potential screening applications.[6] However, one limitation to remain with respect to the beads currently available is the limited loading capacities of most polystyrene and PEG-based resins. This is especially a problem in split and mix synthesis, be it bead or Kan based.[7] As a result, most companies have favoured parallel synthesis over Split and Mix for high-throughput library synthesis, despite the fundamental efficiency of the latter technique. Therefore, finding novel supports with high-loading capacities that allow larger amounts of compound to be released from a single-bead is of great interest.

To date, most research in this area has focused on the use of hyperbranched polyfunctional molecules in a process known as dendrimerisation. Dendrimers are an intriguing new class of molecules with a wide range of potential applications ranging from drug-delivery systems to catalyst carriers.[8] There are two different methods for the synthesis of dendrimers. The divergent approach based on the successive attachment of the branching unit to the core, and the convergent approach where dendrimeric fragments are condensed together. Both methods suffer from difficulties associated with long reaction times and non-trivial purification.[9] Solid-phase methodology enables the use of excess reagents to drive reactions to completion, turning purification steps into simple washings of the resin. In this paper, we report on the evaluation of a 1→3 *C*-branched isocyanate type monomer as a means of loading enhancement. Single-bead loading as well as swelling properties, kinetics and mechanical stability were assessed in order to determine the optimal monomer/generation for synthetic applications.

2. Results and discussion

In 1997 we reported that dendrimer assembly on solid support was a practical method for bead-loading enhancement.[10] Polyamidoamine (PAMAM) dendrimers were synthesised on TentaGel™ resin by cyclic treatment of methyl acrylate followed by 1,3-diaminopropane (displacing the methyl esters). Each new cycle was characterised by the generation of an exponentially increasing number of amino groups on the periphery. Although PAMAM dendrimers have now been successfully synthesised on a diverse number of supports, defect structures arising from intramolecular lactam formation are potential side-reactions.[9]

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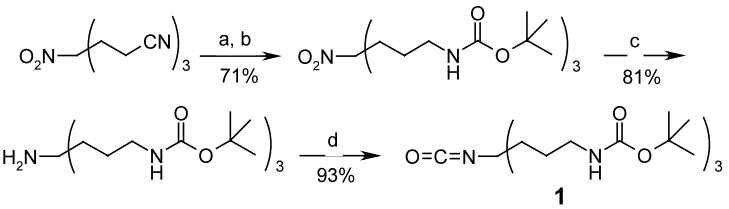
These reactions result in an accumulating number of statistical defect structures reducing the loading capacity of the resin, with the likelihood of defects becoming increasingly significant at higher generations due to steric congestion.

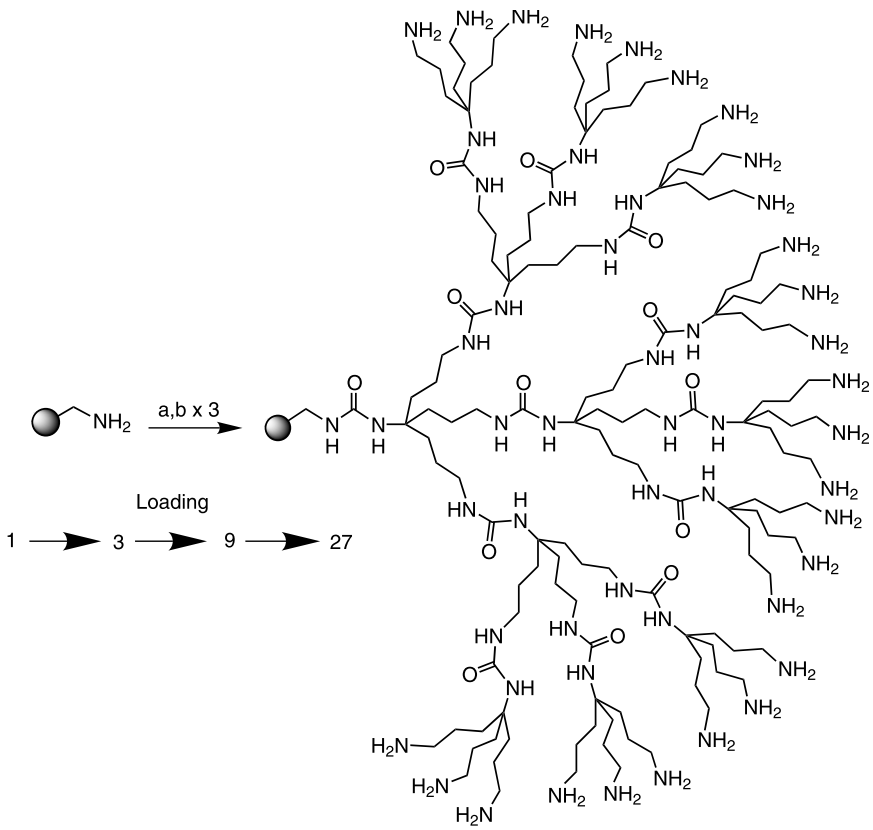
Among the various types of dendritic building blocks available the symmetrical 1→3 *C*-branched isocyanate monomers, developed by Newkome et al., are of interest due to their high branching multiplicity and the presence of a reactive isocyanate moiety which allows this building block to be used for the attachment to amine functionalities and rapid growth of dendrimeric assemblies.[11] In this letter we describe the use of a symmetrical 1→3 *C*-branched isocyanate monomer bearing a triad of protected amines, for resin dendrimerisation. Amine deprotection followed by subsequent reaction with the isocyanate monomer affords the next dendrimer generation without cross-coupling problems and with rapid growth in functionality.

Isocyanate monomer **1** was synthesised using a modification of the procedure reported by Newkome et al.[11] Synthesis was carried out starting with commercially available tris(2-cyanoethyl)nitromethane (Scheme 1). Reduction of the nitrile groups with borane–THF afforded the crude triamine, which was protected with Boc_2O to yield the tris(Boc) protected triamine. Reduction of the nitro group at 1 atm of hydrogen at 80°C for 42 h gave the tris(Boc)amine. Treatment of this sterically hindered amine with DMAP and Boc_2O gave the expected isocyanate **1** in 93% yield.[12]

Solid-phase dendrimer synthesis was carried out on polystyrene aminomethyl resins (250–300 μm, 1.38 mmol/g and 75–150 μm, 0.92 mmol/g) as well as on TentaGel™ resin (160 μm, 0.46 mmol/g) following the procedure depicted in Scheme 2. The amino-derivatised resins were reacted with the isocyanate **1** to afford resin-based dendrimer generation 0.5, which upon treatment with TFA gave generation 1.0. This process was



Scheme 1. Synthesis of 1→3 *C*-branched isocyanate monomer. (a) BH_3·THF, dioxane, reflux, 2 h; (b) Boc_2O, NEt_3, MeOH, reflux, 2 h; (c) H_2, Raney nickel, EtOH, 80°C, 42 h; (d) Boc_2O, DMAP, CH_2Cl_2, 3 h.



Scheme 2. Solid-phase dendrimer synthesis: (a) monomer (**1**), DIPEA, DMAP, DCM and/or DMF; (b) 40% TFA/DCM, washed with 5% DIPEA, DMF.

repeated to afford higher generations. Each coupling was monitored by a qualitative ninhydrin test, and repeated if necessary to drive the reaction to completion. The loading of each full generation was measured by quantitative Fmoc test and bead counting. The results are summarised in Table 1.

A 10-fold increase in loading for standard polystyrene resin and TentaGel™ was determined. However, a major problem was encountered with the larger polystyrene beads. Treatment of generation 1.5 dendrimer-beads with 40% TFA in DCM, while attempting to form generation 2.0 resulted in breakage of the majority of the beads. Observations under the microscope revealed that the damage was caused by bubbles growing within the beads as a result of the formation of carbon dioxide and isobutylene during the deprotection step, as well as the dendrimeric branches trying to position themselves as far apart as possible to minimise electronic repulsion, resulting in rupture of the polystyrene matrix ('umbrella effect'). It was found that progressively increasing the TFA concentration to remove the *tert*-butoxycarbonyl groups could overcome bead breakage during the formation of the second generation dendrimer, giving a subsequent loading of nearly 100 nmol per bead. However, these resin beads were not physically robust enough to accommodate formation of generation 3.0, using the same deprotection procedure.

Swelling studies were carried out on the 75–150 μm polystyrene and 160 μm TentaGel™ resins at each new generation (Fig. 1). The swelling behaviour of dendrimer-bound-TentaGel™-resins was altered compared to TentaGel™. Thus, swelling was very uniform for the third generation materials across all the solvents, with polar solvents being slightly preferred, presumably this is due to the characteristics of the dendrimer dominating bead solvation as well as the fact that bead has in essence already been 'stretched' to accommodate the bulk of the dendrimer. With polystyrene resin the dendrimers attached directly on the polystyrene backbone of the resin dictated the swelling to a much more significant degree. Dendrimerised resins swelled to a greater degree in polar solvents than the initial aminomethyl polystyrene resin, while solvation in more hydrophobic solvents was very limited. This can be rationalised by the fact that when the peripheral amino termini are being well solvated, they extend, whereas in poor solvating solvent, they actually contract and fold back.[13]

Kinetic studies based on Fmoc release were carried out on the large polystyrene beads (250–300 μm) in order to determine the reactivity profile of each resin-bound generation, as well as assessing the optimum dendrimer generation for synthetic applications (Fig. 2).

Deprotection of Fmoc-Gly attached to the periphery of the dendrimer with 20% piperidine in DMF required 22 and 57 min for generations 1.0 and 2.0, respectively, compared to 5 min for the aminomethyl resin alone. This is the consequence of steric congestion interfering with site accessibility.

Generation 1.0 was therefore chosen for synthetic application and used for the synthesis of a tri-peptide and a biaryl compound. Fmoc-Ala-Val-Phe-OH was synthesised via a standard HOBt/DIC procedure using

Table 1. Loading measurements on resin-bound dendrimers

Resin	Initial loading (nmol/bead)	Gen.1.0 (nmol/bead)	Gen. 2.0 (nmol/bead)	Gen. 3.0 (nmol/bead)	Overall loading increase
PS (250–300 μm)	17.9	40.6 (76%)[a]	95.6 (78%)	Broken	×5.3
PS (75–150 μm)	0.66	1.53 (77%)	3.61 (79%)	6.61 (61%)	×10.0
TG (160 μm)	0.68	1.38 (68%)	3.18 (77%)	6.91 (72%)	×10.2

[a] Yields based on theoretical maximum loading.

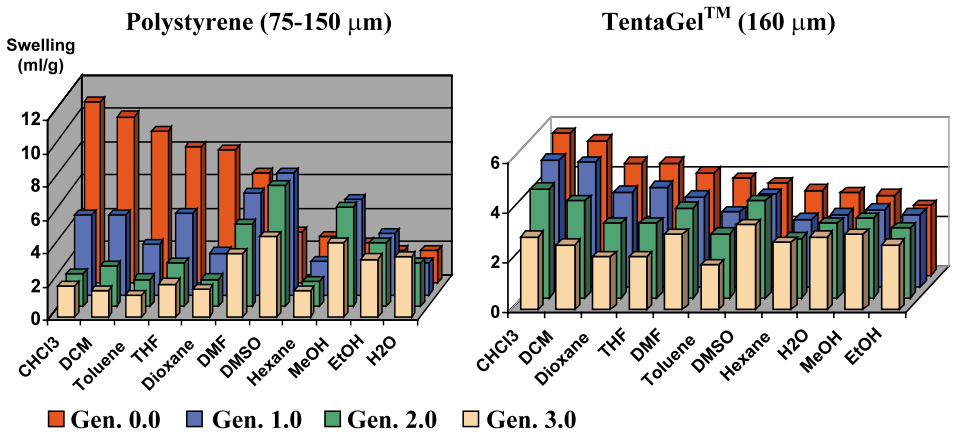


Figure 1. Swelling studies for PS and TentaGel™ dendrimer resins.

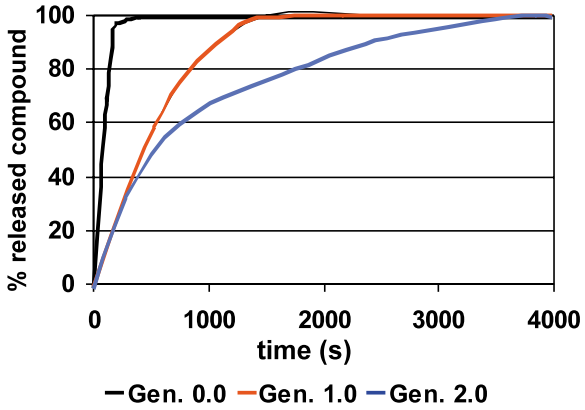


Figure 2. Kinetic studies of Fmoc release from functionalised dendrimer resin.

an HMPB linker, which also acts as a spacer to render the ends of the supported dendrimer more flexible and reduce steric congestion. The tripetide was produced in quantitative yield from the generation 1.0 resin beads with a purity of 100% (as determined by HPLC, $\lambda = 220$ nm). A Suzuki coupling was also investigated coupling 4-iodobenzoic acid onto the HMPB linker followed by treatment with 4-methylbenzene boronic acid coupled under standard conditions. Cleavage afforded the biaryl compound in 59% yield with an HPLC purity of 100%. The beads were robust to all reaction conditions and extensive solvent washing.

In conclusion, we have developed an efficient high-loading resin-bound dendrimer in which a symmetrical tri-branched protected monomer is used for effective dendrimerisation without problems associated with side-reactions or cross-coupling. This resin exhibited good swelling behaviour in polar solvents, including water. Generation 1.0 proved to be an efficient and robust support for synthetic applications.

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