



Resin-bound Dendrimers as High Loading Supports for Solid Phase Chemistry

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Abstract

The synthesis of a *tris*-Boc tri-amino acid and its utility in the generation of dendrimers as inert scaffolds on solid supports is described. These high loading amine functionalized resins were successfully used in the syntheses of small molecules. © 1999 Elsevier Science Ltd. All rights reserved.

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Dendrimer research has evolved into an area of considerable interest in recent years. Dendrimers are highly branched macromolecules built generationally from a central core by stepwise and controlled processes. These dendrimeric structures differ from typical polymers in possessing well defined molecular architectures. Thus, dendrimers have found widespread applications as catalyst carriers, drug delivery systems, complexation agents, *etc.* [1-4].

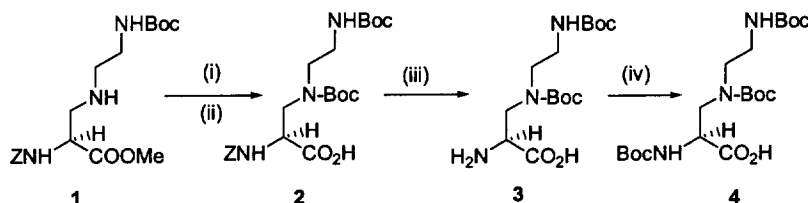
The solution phase synthesis of dendrimers is either by the 'divergent approach' involving repetitive multi-step synthesis [5-7] or by the 'convergent method' where small fragments of the dendrimer are assembled on a multi-functional core [8-10]. However, both these approaches suffer from the disadvantage of repetitive time-consuming purification. In contrast, dendrimer synthesis on solid supports [2,3] makes the purification procedure simpler as excess reagents ensure reaction completion and can be easily washed off from the polymer bound dendrimer molecule.

The naturally occurring amino acid lysine has been used as a building block in the generation of inert dendrimeric scaffolds, which are commercially available for the synthesis of highly immunogenic multiple antigenic peptides [11]. It was anticipated that the unique tri-amino acids described in the previous paper [12] could be exploited for the generation of dendritic scaffolds on solid supports. We envisaged that repeat coupling of the protected tri-amino acid **4** onto an amine functionalized resin would lead to a geometrical increase in the number of reactive amine functionalities giving extremely high loading per reaction bead.

The synthesis of **4** was readily accomplished by acylating the secondary amine

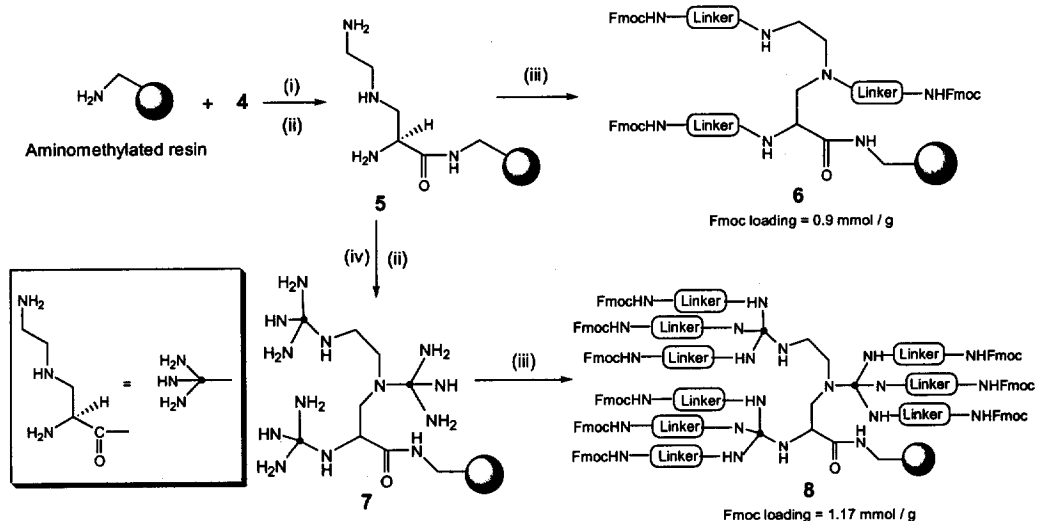
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functionality in the tri-amino ester **1** with di-*tert*-butyl dicarbonate followed by saponification of the methyl ester to give **2**. Catalytic hydrogenolysis gave the *bis*-Boc amino acid **3**. Finally, acylation of **3** at the α -amino position by treatment with $(\text{Boc})_2\text{O}$ in presence of triethylamine afforded the required *tris*-Boc tri-amino acid **4**¹ (Scheme 1). Attempts to synthesize **4** from **2** by one-pot catalytic hydrogenolysis in the presence of $(\text{Boc})_2\text{O}$ was not successful as the product obtained was heavily contaminated with the N^α, N^α -diBoc derivative of **3**.



Scheme 1 Reagents and conditions : (i) $(\text{Boc})_2\text{O}$; (ii) NaOH, MeOH–water; (iii) H_2 / 10% Pd-C, MeOH; (iv) $(\text{Boc})_2\text{O}$, Et_3N .

The *tris*-Boc amino acid **4** was readily attached to aminomethylated PS resin (0.8 mmol/g) preswollen in DMF, *via* the DIPCDI–HOAt mediated coupling (Scheme 2). Complete removal of the three Boc protecting groups was effected by treatment of the above resin with 50% TFA–DCM in the presence of TES to give **5**, the first generation amines.



Scheme 2 Reagents and conditions : (i) HOAt–DIPCDI, DMF; (ii) 50% TFA–DCM, TES; (iii) Rink linker, HOAt–DIPCDI, DMF; (iv) **4**, HATU–DIPEA, DMF.

¹ Spectral data of N^2, N^4, N^6 -*tris-tert*-butoxycarbonyl-2(*S*), 6-diamino-4-azahexanoic acid **4**:

ν_{max} 1701 (C=O), 3373 (NH) cm^{-1} ; δ_{H} (250 MHz, CDCl_3) 1.41, 1.42, 1.45 (27 H, 3 x s, 3 $(\text{CH}_3)_3$), 2.85–3.89 (6 H, m, 3 CH_2), 3.90–4.78 (1H, m, α -H), 4.88–5.50 (1 H, m, NH), 5.66–6.40 (1H, m, NH), 8.57 (1 H, br s, COOH); δ_{C} (62.9 MHz, CDCl_3) 28.18, 28.27 (3 $(\text{CH}_3)_3$), 39.17 (CH_2), 47.88 (CH_2), 48.95 (CH_2), 52.69 (CH), 79.46, 79.97, 80.94 (3 $\text{C}(\text{CH}_3)_3$), 155.85 (3 $\text{COC}(\text{CH}_3)_3$), 173.33 (COOH); m/z (FAB) 470 (M+Na), 448 (M+H); HRMS (FAB) 470.2472 (M+Na, $\text{C}_{20}\text{H}_{37}\text{N}_5\text{O}_8\text{Na}$ requires m/z 470.2478), 448.2656 (M+H, $\text{C}_{20}\text{H}_{37}\text{N}_5\text{O}_8$ requires m/z 448.2659).

The Rink amide linker [13] was then coupled onto resin **5** to give the derivatised PS resin **6**. The efficiency of all coupling steps and deprotections was monitored using the TNBS amine test [14]. Significantly, the resin **6** exhibited substitution of 0.9 mmol/g (89%) based on spectrophotometric determination of the Fmoc-derived chromophore liberated upon treatment with 20% piperidine in DMF using $\epsilon_{290} = 5253 \text{ M}^{-1} \text{ cm}^{-1}$.

Furthermore, resin **5** was re-exposed to the *tris*-Boc amino acid **4** using standard coupling conditions, which when followed by TFA treatment gave the second generation amines **7**. Attachment of the Rink linker to the resin **7** using standard coupling conditions was monitored by a negative amine test. Once again, the resin **8** exhibited an excellent loading of 1.17 mmol/g (84%) based on UV analysis of Fmoc deprotection product, dibenzofulvene-piperidine adduct.

Thus, the high loading dendrimeric resins of 1st and 2nd generation amines were readily obtained, particularly coupling of the linker at the secondary amine functionality was easily achieved. Although the loading per gram of the resin does not increase substantially due to the mass of the compound attached to the resin, the loading per reaction bead increases geometrically from one generation to the next.

To assess the physical appearance of the resin beads, the unmodified aminomethylated resin beads and the second generation dendrimer beads were swollen in DCM and analyzed under an optical microscope with 100 x magnification (Figure 1). The second generation dendrimer beads are clearly larger in size compared to the parent aminomethylated polystyrene.

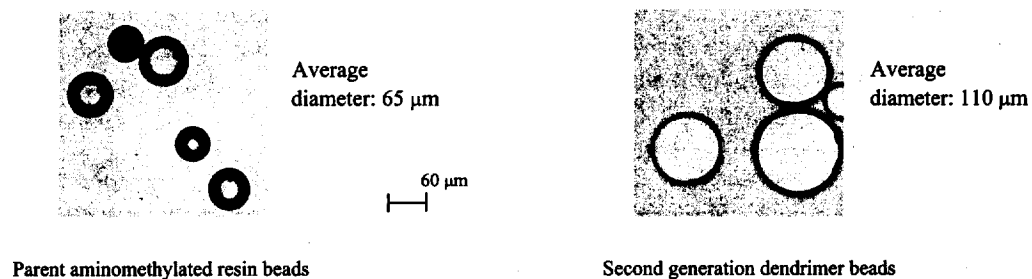
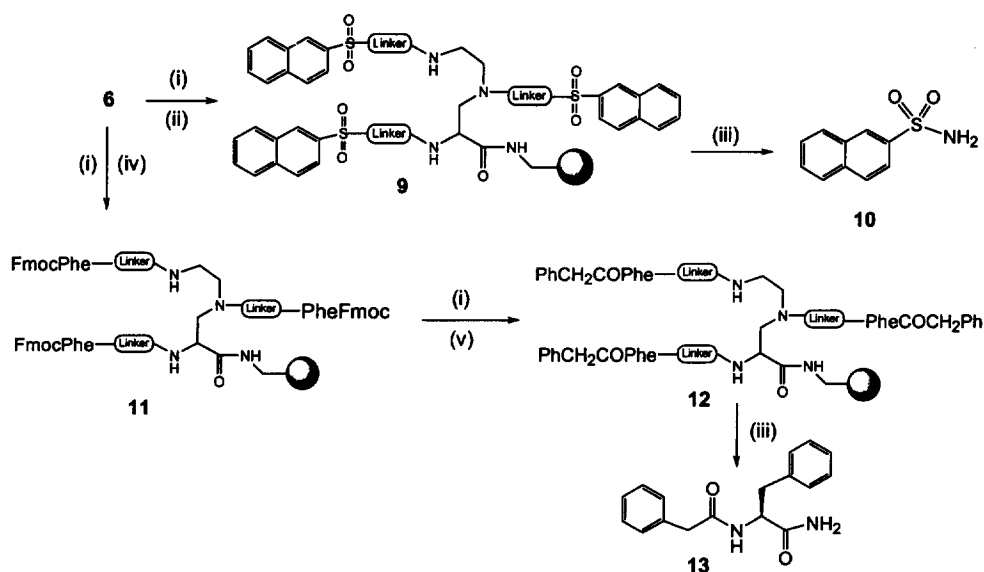


Figure 1

The high loading efficiency of these dendrimeric resins was further evaluated by the synthesis of 2-naphthalenesulphonamide **10** and *N*-phenylacetyl-L-phenylalaninamide **13**. Thus, resin **6** was subjected to Fmoc deprotection, followed by reaction with 2-naphthalenesulphonyl chloride in the presence of DIPEA to give the derivatised resin **9**. 2-Naphthalenesulphonamide **10** was then selectively cleaved from the resin by treatment with 90% TFA-water in 84% yield, leaving the dendrimer core intact (Scheme 3). The identity and purity of **10** was confirmed by ES-MS, ¹H and ¹³C NMR.

For synthesis of compound **13**, the resin **6** was treated with 20% piperidine-DMF followed by acylation with FmocPheOH using standard coupling conditions. The resin **11** showed a loading of 0.9 mmol/g. Fmoc-removal followed by derivatisation with phenylacetic acid afforded resin **12**. Compound **13** was then released as before in 87% yield.

Moreover, the above sequences were repeated on the 2nd generation amines and the compounds **10** and **13** were obtained in near quantitative yields on cleavage.



Scheme 3 Reagents and conditions : (i) 20% piperidine/DMF; (ii) 2-naphthalenesulphonyl chloride, DIPEA, DMF; (iii) 90% TFA/water; (iv) FmocPheOH, HATU, DIPEA,DMF; (v) PhCH₂CO₂H, HOBt, TBTU, DIPEA.

In summary, we have demonstrated the construction of a new class of resin-attached dendrimers by using our novel *tris*-Boc tri-amino acid as a building block. These dendrimers provide a rapid and efficient method of increasing resin loading. Furthermore, we envisaged that *via* orthogonal protection on the three amine functionalities, it is possible to devise a multi-release system for generation of one-bead-one-compound libraries.

Abbreviations Boc, *tert*-butoxycarbonyl; DIPCPI, *N,N'*-diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DCM, dichloromethane; DMF, *N,N'*-dimethylformamide; FAB, fast-atom-bombardment; Fmoc, 9-fluorenylmethoxycarbonyl; HOAt, 1-hydroxyazabenzotriazole; HOBt, 1-hydroxybenzotriazole; TBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TES, triethylsilane; TFA, trifluoroacetic acid; TNBS, 2,4,6-trinitrobenzenesulfonyl acid; Z, benzyloxycarbonyl.

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