Solid phase synthesis of aryl-ether dendrimers

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Solid phase chemistry can be used to prepare, in excellent yield and purity, a range of Fréchet-type aryl-ether dendrimers, for use in either dendrimer conjugation studies, resin loading enhancement or the wedge based synthesis of larger dendrimers.

Over the past few years we have shown that resin-bound polyamidoamine (PAMAM) dendrimers are readily synthesized[1,2] and are inert under a broad range of chemical conditions[3] such as coupling reactions, nucleophilic displacements or borohydride reductions. However, synthetic patterns requiring the use of strong reducing agents or strong bases have to be avoided. In the area of solution dendrimer synthesis Fréchet[4] has reported the construction of arylether dendrimers *via* the alkylation of bromobenzylic dendrimeric fragments with 3,5-dihydroxybenzyl alcohol, with which he also introduced the concept of convergent dendrimer synthesis. Since polyether dendrimers are substantially more chemically inert than the PAMAM dendrimers and because of the problems associated with solution dendrimer synthesis, the solid-phase synthesis of Fréchet-type aryl-ether dendrimers (Fig. 1) was targeted both as a means of resin loading enhancement as well as a convenient approach to dendrimer *wedge*[4] synthesis. Since the building blocks originally used by Fréchet were considered to be unsuitable for solid-phase synthesis, 3,5-bis(acetoxymethyl)-phenol (**1**) was used and was prepared by modification of the literature procedure.[5,6] The presence of a phenolic group and of two acetyl-protected primary alcohols allowed the synthesis of the dendrimer *via* a two-step iterative procedure, consisting of a Mitsunobu condensation followed by ester hydrolysis. The synthesis was first carried out using an analytical construct,[7] consisting of glycine attached to the TFA-cleavable Rink linker[8] allowing ready characterisation of the intermediates and optimisation of the Mitsunobu condensations and ester hydrolysis. Compound **2**, obtained in two steps from **1**, was coupled onto Gly-Rink-PS resin under standard conditions (Scheme 1) to give **3**.

Hydrolysis of **3** followed by Mitsunobu condensation with (**1**) afforded Generation 2.0 dendrimer after TFA cleavage. The most efficient conditions for the hydrolysis were found to be Bu_4NOH(aq)–THF 1:3, while the Mitsunobu reaction was performed with DIAD–PPh_3† in THF. Generation 3.0 and 4.0 dendrimers were synthesized using the same procedure and the crude HPLC trace of Generation 3.0 dendrimer is shown in Fig. 2.[9] Dendrimers were subsequently synthesized directly on hydroxymethylpolystyrene **4** (Scheme 2), which was obtained from commercial Merrifield resin (1% DVB, 0.93 mmol g^{-1}, 90–106 μm) *via* displacement of the chloride with caesium acetate in DMF and hydrolysis of the resulting ester with Bu_4NOH(aq) in THF. This was extended to give dendrimer resin **6** as shown in Scheme 2.

The loading of resin **4** was determined to be 0.82 mmol g^{-1} (0.44 nmol $bead^{-1}$). Synthesis of generation 3.0 dendrimer resin gave a resin with a loading of 2.85 mmol g^{-1} (3 nmol

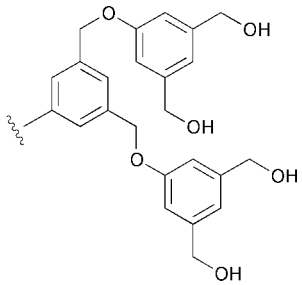
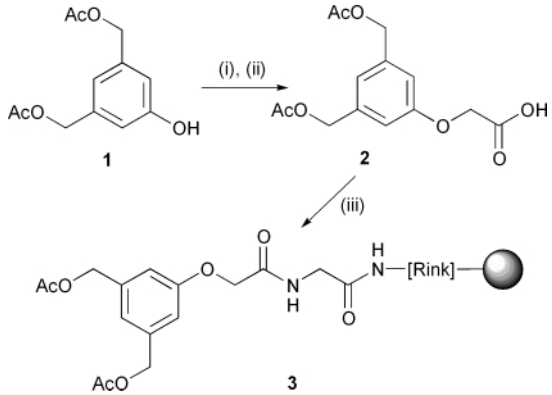


Fig. 1 Fréchet-type aryl-ether dendrimer fragment.



Scheme 1 (i) *tert*-Butylbromoacetate, K_2CO_3, CH_3CN; (ii) TFA, DCM (2:3), (iii) H-Gly-Rink-PS, DIC,† HOBt, DMF.

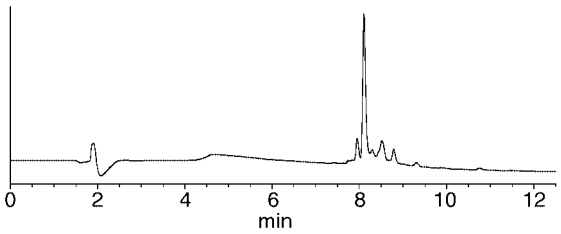
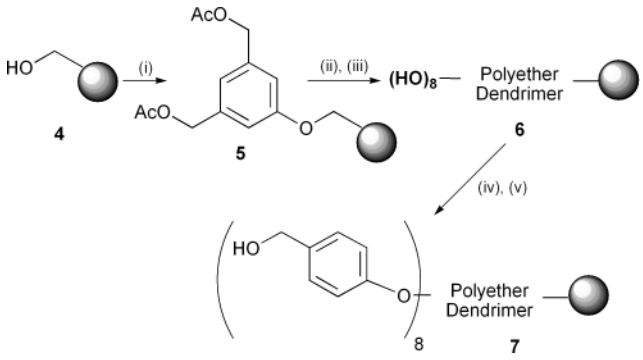


Fig. 2 HPLC trace of polyether dendrimer Generation 3.0.



Scheme 2 (i) **(1)**, DIAD, PPh_3, THF; (ii) Bu_4NOH (aq), THF (1:3); (iii) Repeat twice (i) and (ii), (iv) methyl-4-hydroxybenzoate, DIAD, PPh_3, THF; (v) $LiAlH_4$, THF.

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Table 1 Bead diameter (μm) in different solvents (average of 10 beads)

	Dry	DCM	DMF	MeOH	H_2O	THF
4	90	165	155	95	95	175
6	110	145	185	105	110	150
$(Ac)_8$-6	115	195	190	110	115	190

bead^{-1}) (7 times that of the initial resin). Since the synthesis of this dendrimer involved 7 distinct Mitsunobu condensations and 14 distinct ester hydrolyses and the final loading was 86% of that expected this implies that each reaction had proceeded to 99.3% completion. The swelling properties of this resin were analyzed and compared with those of hydroxymethylpolystyrene and are summarized in Table 1.

The diameter of the dry resin beads increased by about 25% after the dendrimerisation process. Acetyl-protected dendrimer resin had the same swelling trend as the hydroxymethylpolystyrene **4**, while resin **6** presented some interesting features with apolar solvents like DCM and THF. In these solvents the resin swelled much less than the parent acetyl-protected resin and also less than resin **4**, due to the high density of hydroxy groups. The two resins **4** and **6** were also compared in terms of reaction kinetics. Fmoc-Ala-OH was coupled onto the two resins and Fmoc and quantitative ninhydrin tests were carried out with samples removed over 2 h. Differences in reactivity were found to be negligible.

To prove the versatility of this new resin, methyl 4-hydroxybenzoate was coupled *via* a Mitsunobu condensation on to resin **6** and the resin-bound methyl ester was then reduced with $LiAlH_4$ (Scheme 2). This two-step procedure was found to be an efficient alternative method to introduce the Wang linker onto a polystyrene support. The polyether dendrimer resin was perfectly stable to $LiAlH_4$ reduction and the final loading of the dendrimer–Wang resin **7** was found to be 2.3 nmol bead^{-1}. The utility of resin **7** was demonstrated by synthesising the hexapeptide Leu-Enkephaline-Lys. Following cleavage with TFA–H_2O 95:5 and purification, the peptide was isolated in 66% yield, relative to the loading of resin **4**.

In conclusion, we have demonstrated that the polyether dendrimer resin can be conveniently and efficiently synthesised on hydroxymethyl polystyrene resins and that the final loading is sufficient for multiple single-bead screenings and is inert under severe chemical conditions. Moreover we have shown that polyether dendrimers can be conveniently synthesised on the solid phase. No purification of the intermediates (usually a non-trivial step, taking in account the molecular weight and character of these molecules) is required to obtain highly pure compounds. The *wedges* synthesised on solid phase could be used to assemble bigger fragments according to the convergent dendrimer methodology outlined by Fréchet.

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Notes and references

† DIAD = diisopropylazodicarboxylate. DIC = diisopropylcarbodiimide.

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9 NMR of Generation 3.0 dendrimer: DMSO-d_6: $\delta(^1H)$ = 7.30 (s, 1H, ArH Gen 1.0), 7.24 (s, 2H, ArH Gen 2.0), 7.19 (br s, 6H, ArH Gen 1.0 and 2.0), 7.06 (br s, 2H, NH_2), 6.97 (s, 4H, ArH Gen 3.0), 6.95 (s, 8H, ArH Gen 3.0), 5.22 (s, 4H, CH_2 Gen 1.0), 5.17 (s, 8H, CH_2 Gen 2.0), 4.68 (s, 2H, O-CH_2-CO), 4.55 (s, 16H, CH_2 Gen 3.0), 3.85 (br s, 2H, NH-CH_2-CO); $\delta(^{13}C)$: 171.1, 168.3, 159.0, 158.8, 158.4, 144.3, 139.5, 139.3, 120.0, 119.3, 117.4, 113.8, 113.5, 111.4, 69.5, 69.3, 67.4, 63.2, 41.8; *m/z* (TOF LD$^+$): 1107.5 (100%, $(M + Na)^+$), 1123.4 (30%, $(M + K)^+$).