Solid-phase synthesis of a lysine-capped bis-dendron with remarkable DNA delivery abilities†

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Solid-phase synthesis of a generation 3.0 polyamidourea 1→3 *C*-branched bis-dendron followed by capping of the peripheral amino groups with L-lysine gave an efficient transfection reagent.

Polymers have emerged as a vital component of many medical technologies and as a result demands have arisen for the adaptation of the size, topology, chemistry and properties of a multitude of polymeric materials.[1,2] In this arena, dendrimers have acquired a privileged status, firstly because of their unique structural features as highly branched, symmetrical, monodisperse polymers, and secondly due to their tunable chemistry, which allows the production of compositionally and structurally controlled macromolecules.[3]

Polycationic dendrimers have been widely investigated as vectors for drug delivery and release,[1,4–6] and also as devices for efficiently transfecting cells (*e.g.* SuperFect, PAMAM derivatives, *etc*).[7–11] However, despite all these efforts, the mechanisms and structural requirements which enable DNA–dendrimer complexes (also called dendriplexes) to cross the cellular membrane,[12] enter the cytoplasm and then release their cargo are still unclear. A number of investigations have demonstrated that the high-generation polycationic dendrimers (*e.g.* PAMAM G >5, containing 128 primary amines + 126 tertiary amines) show efficient transfection properties.[13] However, as the dendrimer generation increases, so does cytotoxicity, while difficulties in obtaining pure compounds increases proportionately.[1,13] What would be highly desirable would be a synthetically accessible, low-generation, yet highly efficiently transfecting dendrimer.

In this manuscript,[14] the solid-phase synthesis and remarkable transfection abilities of a G 3.0 polyamidourea 1→3 *C*-branched bis-dendron are reported, where the peripheral amino groups of the structure have been capped with L-lysine.[15] The dendritic structures were synthesized using a divergent, microwave-assisted, solid-phase approach with the dendrimer assembled on polystyrene resin *via* an acid labile linker.[16] The acid-cleavable polyamine scaffold **4** was used as a core for assembling the dendrons (prepared as previously reported[16,17]) and attached onto the solid support to give **5** (see Scheme 1). The dendrimer was constructed (up to G 3.0) by the sequential addition of the AB_3 isocyanate-type monomer **6**[19] under microwave irradiation conditions, followed by the displacement of the methyl ester with propane-1,3-diamine (see Scheme 2). The use of tris-branched monomer **6** leads to a rapid increase in terminal functionality (2 → 6 → 18 → 54),[16] with acid treatment of resin-bound dendrimer **7** giving G 3.0 bis-dendron **8** (see Fig. 1). Treatment of **7** with Fmoc-Lys(Boc)-OH/DIC/HOBt followed by Fmoc-deprotection and resin cleavage gave dendrimer **9**, with ^{1}H-NMR analysis (see characterization in the ESI†) suggesting that approximately 50 out of the 54 terminal amino groups had been

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† Electronic supplementary information (ESI) available: General chemistry procedures, experimental details for the synthesis, characterization of the novel compounds, and biological assay protocols. See DOI: 10.1039/b804771e

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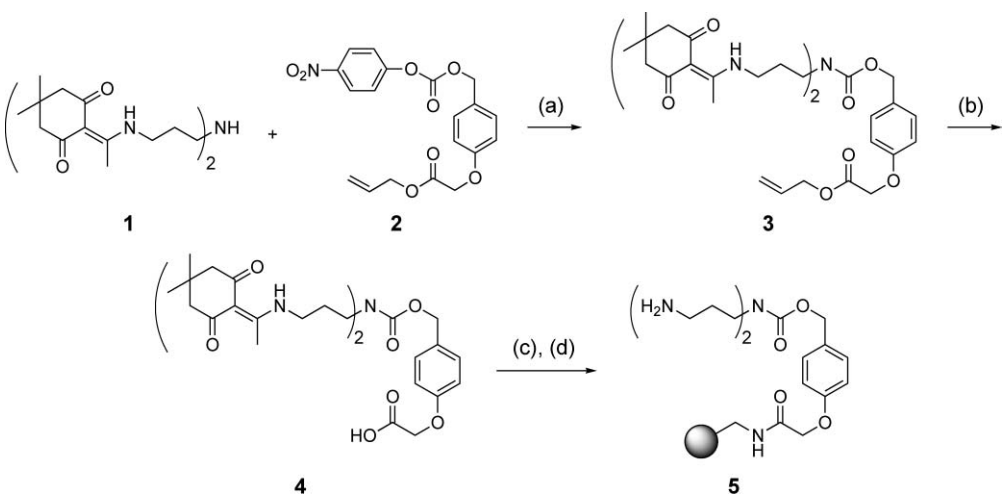
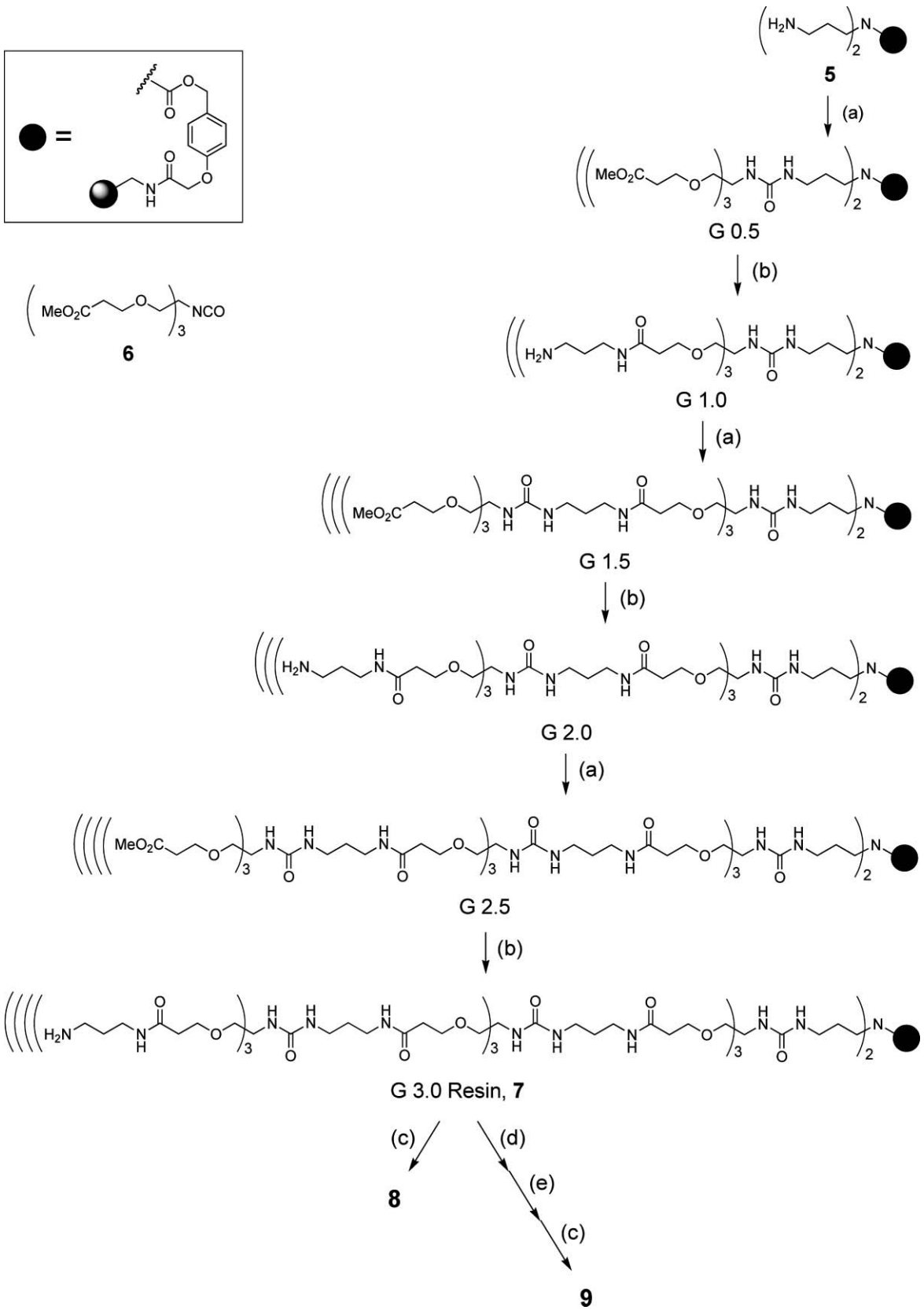


Scheme 1 *Reagents and conditions*: (a) NEt_3, DMAP, DMF, 81%; (b) polymer-supported Pd,[18] pyrrolidine, THF, reflux, 94%; (c) aminomethyl resin, DIC, HOBt, DCM; (d) 5% $N_2H_4{\cdot}H_2O$ in DMF.



Scheme 2 *Reagents and conditions*: (a) monomer **6**, DMAP, DIPEA, DMF–DCM 1 : 1, μW, 100 °C, 60 min; (b) propane-1,3-diamine, MeOH, 72 h; (c) TFA–DCM–H_2O (9.5 : 0.25 : 0.25), 3 h; (d) Fmoc-Lys(Boc)-OH, DIC, HOBt, DCM–DMF 1 : 1, overnight; (e) 20% piperidine in DMF, 2 × 30 min.

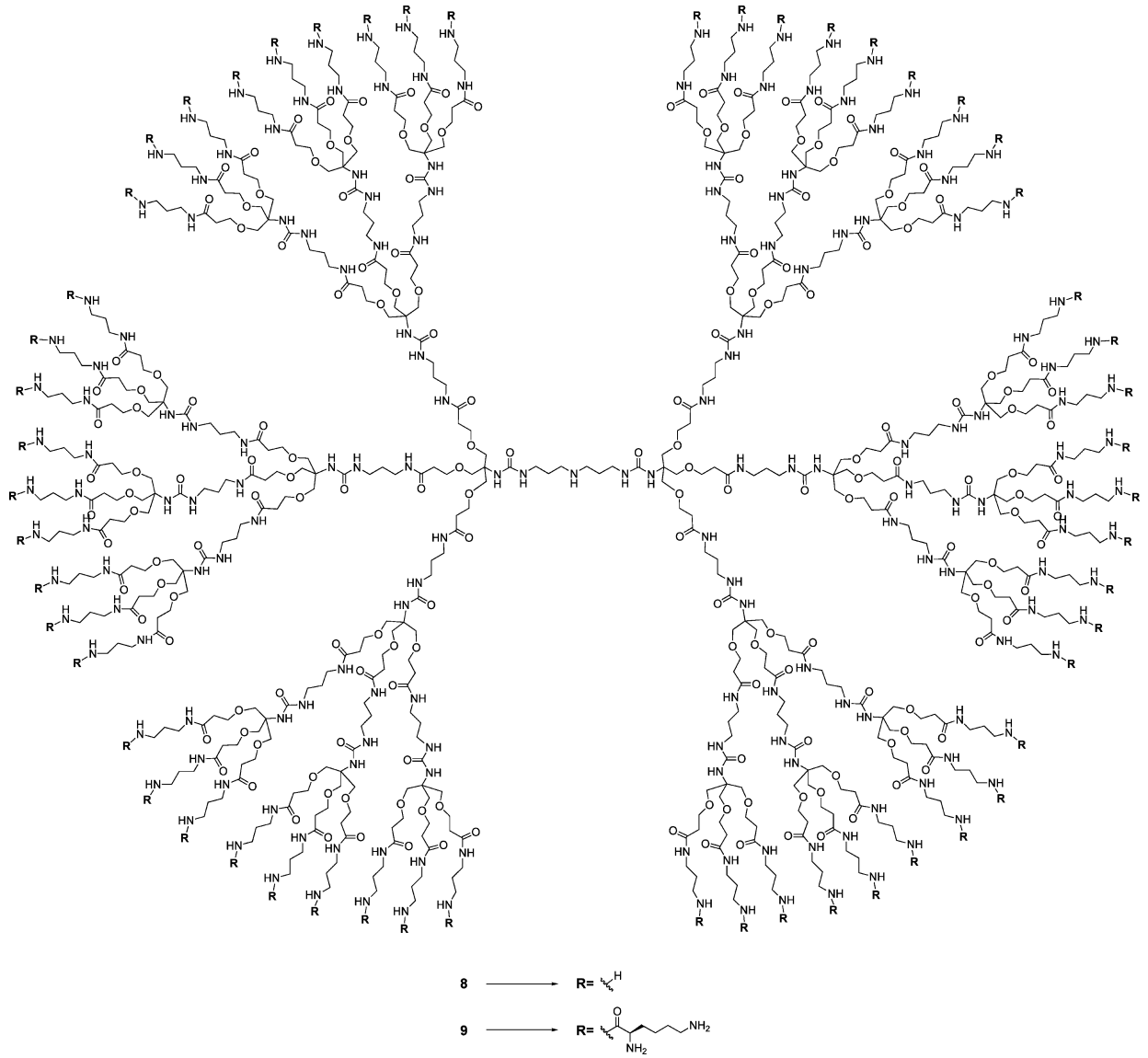


Fig. 1 Structures of G 3.0 polyamidourea 1→3 *C*-branched bis-dendrons **8** and **9**.

coupled (attempts to force the reaction further were not successful despite the use of prolonged microwave heating).

The ability of the dendrimers to complex DNA was studied by conventional electrophoretic DNA retardation assays. Dendriplexes were formulated as a function of dendrimer/DNA weight ratios, and representative electrophoretic gel images are shown in Fig. 2, showing that dendrimers **8** and **9** completely suppressed the electrophoretic mobility of plasmid DNA (this was also observed for the positive controls SuperFect and Effectene).

The transfection properties of dendrimers **8** and **9** were evaluated with four different mammalian cell lines (HEK293T, HeLa, ND7, and B16F10) employing pEGFP-N1 as a GFP-reporter. Dendriplexes were formulated at two weight ratios (10 : 1 and 20 : 1) and tested in triplicate using SuperFect (a dendrimeric material) and Effectene (a lipid formulation) as controls, with GFP expression evaluated by flow cytometry.[20]

As observed in Fig. 3, dendrimer **9** showed high gene delivery efficacy with HEK293T, ND7 and B16F10 cells. GFP expression

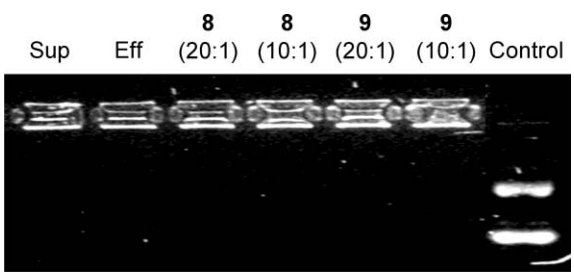


Fig. 2 Electrophoretic DNA retardation assays. Dendrimers **8** and **9** were complexed with pEGFP-N1 at weight ratios of 10 : 1 and 20 : 1, loaded onto an agarose gel (1% agarose, 1 μg mL^{-1} ethidium bromide) and run at 100 V for 1 h. Positive controls (Sup = SuperFect; Eff = Effectene) were formulated as suggested by the supplier. Control = naked pEGFP-N1 (containing both supercoiled and nicked circular forms).

was especially high in B16F10 cells at a weight ratio of 20 : 1, with 83% of the cells having fluorescence above background

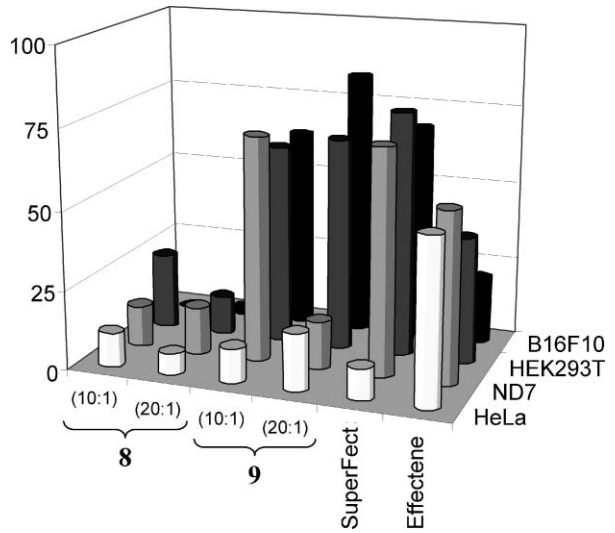


Fig. 3 Percentage of GFP-expressing cell population.

(see ESI† for the flow cytometry data), better than either SuperFect (68%) or Effectene (21%). Since B16F10 (melanoma) cells are considered to be a hard-to-transfect cell line,[21] this result highlights the potential of this reagent. Flow cytometry analysis of neuron-derived ND7 cells showed that dendrimer **9** efficiently delivered pEGFP-N1 into this cell line at a 10 : 1 weight ratio with 71% efficiency. Poor transfection efficiency was found in HeLa cells, consistent with previous work,[12,22] which demonstrated that dendriplexes use a caveolin-dependent pathway as a preferential internalisation route[12] and that HeLa cells are poor at caveolin-dependent endocytosis.[22]

It was interesting to observe that the transfection properties of **8** (displaying 54 amino groups) was insignificant compared to the L-lysine derivative **9** (with 108 amino groups on the surface), clearly indicating that charge density and size are important parameters in transfection with dendriplexes (**8** and **9** were found to be non-toxic at the weight ratios used in all the cell lines tested as determined by MTT assay (>90% cell viability)).

In conclusion, solid-phase methodology in association with microwave heating was used to efficiently construct a G 3.0 polyamidourea 1→3 *C*-branched bis-dendron that, following capping with L-lysine, led to a dendrimer with approximately 108 primary amines. This dendrimer showed remarkable transfection abilities in various mammalian cell lines, comparable or better than SuperFect, a commercially available dendrimer-type transfection reagent.

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