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Self-assembled oligonucleotide-polyester dendrimers†

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A new approach to the controlled synthesis of multi-component dendrimers is presented, in which three oligonucleotide–dendron conjugates were synthesized using solid phase techniques and hybridized to create a second generation polyester dendrimer with DNA as a core and bearing two types of peripheral functional groups.

Dendrimers have emerged as exceptionally versatile scaffolds for the construction of well-defined nanoscale materials. Through the selective introduction of diverse functionality at the core and periphery of these macromolecules, components have been prepared for a variety of applications, including light harvesting systems,[1] drug delivery vectors,[2,3] and lithographic resists.[4] A key next step in this field is the integration of multiple dendritic components into complex, multifunctional molecular structures; however, the high synthetic demand of these materials places limitations on the quantity and types of functional groups that can be incorporated, as orthogonal protecting group strategies or statistical coupling reactions are required to introduce multiple functional domains.[5–8] To address this objective, many research groups have explored self-assembly as an efficient strategy to combine preformed dendritic molecules, typically through electrostatic, hydrogen bonding, and metal–ligand binding interactions.[8–10] However, few of these strategies are capable of combining disparate components in a programmable fashion.

Deoxyribonucleic acid (DNA) is an attractive framework for the construction of self-assembling nanoscale assemblies due to its hybridization fidelity and well-defined double helical structure.[11] The sequence specificity of DNA hybridization allows several strands to be linked in a predictable fashion, leading to complex, highly functional networks. This potential has resulted in the use of DNA scaffolds for the selective arrangement of nanoparticles[12] and proteins.[13] In an effort to explore the use of DNA scaffolds for the organization of dendrimer-based materials through self-assembly, we describe herein a new synthetic strategy for the preparation of DNA–dendron conjugates that retain their hybridization capabilities, and the subsequent construction of multicomponent dendrimers.

As an initial target, a three component system was designed and synthesized to create a dendrimer bearing multiple functional moieties at the periphery (Fig. 1). Since DNA oligomers of any sequence are readily available through the use of automated solid phase synthesis, we sought to develop a similarly convenient synthetic protocol to attach dendritic components before cleaving the oligonucleotides from the controlled pore glass support. The oligonucleotide sequences were chosen so that the two 16 base conjugates, **5d** and **6d**, are fully complementary to the third, **7d**, which is composed of 32 nucleotides. Although many dendritic building blocks are available, the polyester dendrons depicted in Fig. 1 were chosen due to the their facile synthesis and high degree of water solubility when substituted with hydrophilic peripheral groups.

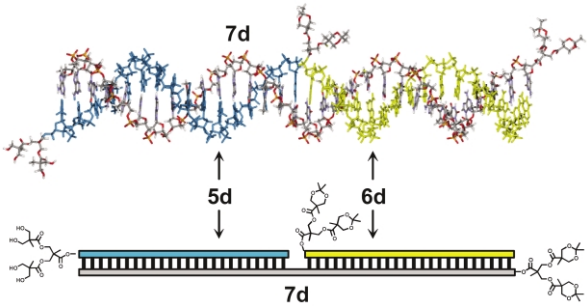
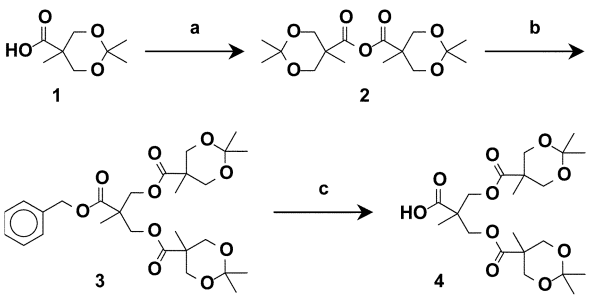


Fig. 1 Three-component oligonucleotide–polyester dendrimer.

These dendritic components, derived from 2,2-bis(hydroxymethyl)propionic acid, were synthesized by first reacting two equivalents of isopropylidene-protected monomer **1**[14] with one equivalent of dicyclohexylcarbodiimide (DCC) to generate anhydride **2** (Scheme 1). Subsequent acylation of the terminal hydroxys of benzyl 2,2-bis(hydroxymethyl)propionate[14] with **2** afforded doubly protected dendron **3**. The benzyl ester core was selectively unmasked under hydrogenolysis conditions to yield **4**, a second generation building block bearing a carboxylic acid focal point.

The oligonucleotide–dendron conjugates were synthesized directly on a controlled pore glass (CPG) solid support. Oligonucleotide sequences were first prepared using an Expedite 8909 DNA synthesizer (Applied Biosystems) with the terminal 5′ dimethoxytrityl (DMT) protecting group retained. The CPG beads were subsequently transferred to a fritted tube (BioRad) and thoroughly rinsed before removal of the DMT protecting group with three exposures to 3% trichloroacetic acid (TCA) in dichloromethane, Fig. 2. A simple protocol was next developed for the acylation of the resulting 5′ hydroxy group with **4**; a 2 h exposure to *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) and dimethylaminopyridine (DMAP) in CH_2Cl_2 was found to be optimal in this regard, affording the supported isopropylidene protected conjugates, **5b**–**7b** (Scheme 2). To generate a conjugate with an alternate peripheral functionality, the acetal periphery was deprotected through exposure to 10% TCA in ethylene glycol to yield **5c**. These



Scheme 1 Synthesis of a polyester dendron building block. (**a**) DCC, CH_2Cl_2, 97%. (**b**) Benzyl 2,2-bis(hydroxymethyl)propionate, DMAP, pyridine, CH_2Cl_2, 94%. (**c**) 10% Pd/C, H_2, ethyl acetate, 95%.

† Electronic supplementary information (ESI) available: experimental section and MALDI-TOF MS. See http://www.rsc.org/suppdata/cc/b2/b209029e/

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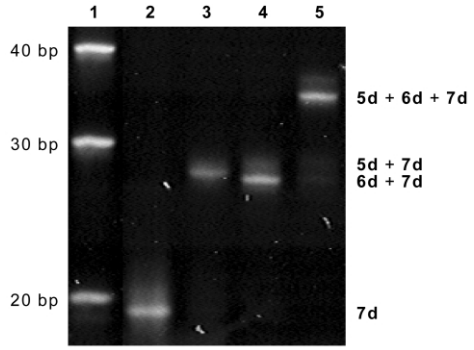
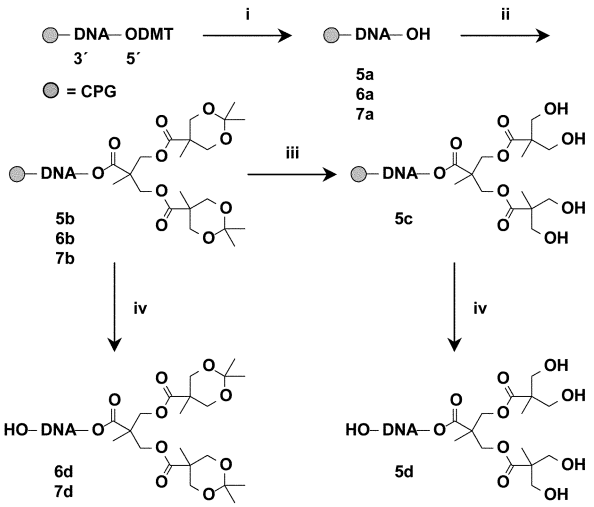


Fig. 2 Native polyacrylamide gel electrophoresis of self-assembled dendrimers. Lane 1: Double stranded DNA ladder (20, 30, 40 bp). Lane 2: **7d**. Lane 3: **5d** + **7d**. Lane 4: **6d** + **7d**. Lane 5: **5d** + **6d** + **7d**. Oligonucleotides were visualized using GelStar stain (BioWhittaker).



Scheme 2 Solid phase synthesis of oligonucleotide–dendron conjugates. (i) 3% TCA/CH_2Cl_2. (ii) 4, DMAP, EDC, CH_2Cl_2 (iii) 10% TCA/ethylene glycol. (iv) Morpholine/methanol (1:1), 60 °C. **5a–d**: DNA (5′-3′) TTCTCTTCAGTTCACA. **6a–d**: DNA (5′-3′) GCAGACGGTAATGACG. **7a–d**: DNA (5′-3′) CGTCATTACCGTCTGCTGTGAACTGAAGAGAA. The shaded sphere represents the controlled-pore glass support.

conditions were uniquely effective for the complete removal of the acetal protecting groups without leading to depurination of the oligonucleotide strand. The peripheral hydroxy groups liberated by this procedure provide reactive sites that can be further functionalized using phosphoramidites or additional acylating agents.

The final step in the conjugate synthesis was the cleavage of the oligonucleotide from the solid support and concomitant removal of the nucleotide base and phosphate protecting groups. The typical ammonium hydroxide conditions used for this purpose could not be employed, as they readily cleaved the ester moiety linking the dendron to the DNA strand. However, a 2 h exposure to a 1:1 mixture of morpholine/MeOH at 60 °C was found to remove all of the protective groups from the oligonucleotide strand and cleave the material from the synthesis support without interfering with the pivaloyl ester linkage. Using this protocol, conjugate **5d** was obtained with four hydroxy groups at the periphery of the dendron. Both **6d** and **7d** were cleaved from the CPG beads prior to acetal removal, providing conjugates with isopropylidene functionalized dendrons.

Purification of the crude conjugates was accomplished by reverse phase HPLC; the collected fractions were analyzed by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Table 1) for characterization. All obtained molecular weights agreed with the calculated values to within 0.08%.

Table 1 MALDI-TOF MS Data for dendrimer building blocks

Conjugate	MW (theory)	MW (exptl.)	Error
5d	5130.5	5126.3	0.08%
6d	5383.8	5380.3	0.07%
7d	10277.0	10271.1	0.06%

To demonstrate the self-assembly ability of the obtained conjugates, the three compounds were combined in equimolar ratios in aqueous 50 mM NaCl solution. The solutions were allowed to anneal at room temperature for 12 h, and the resulting structures were analyzed by non-denaturing polyacrylamide gel electrophoresis (Fig. 2). The single strand conjugate **7d** is shown in lane 2. Upon hybridization with either **5d** or **6d**, the mobility of the duplex band is reduced (lanes 3 and 4, respectively). Complexation of **7d** with both 16-mers produces a trimer of 32 base pairs, as can be seen in lane 5. The migration of the trimer is slower than expected for an unmodified 32-mer due to the presence of the three dendrons on the duplex. The distinct major band in this sample indicates hybridization of the conjugates to give the desired dendrimer target in high yield.

The self-assembly of dendronized oligonucleotides streamlines the synthetic demands required to incorporate multiple functional groups on the periphery of a single molecular target. Through variation of the sequences and lengths of the DNA strands, the structure and functional properties of the assembled dendrimers can be tuned. Furthermore, due to the solid-phase nature of the synthesis, dendrimers bearing more complex functionality can be envisaged, and indeed, we have already successfully attached higher generation dendrimers, chromophores, and polymers to the periphery of these molecules. Current efforts involve the exploration of the physical properties of these materials and the continued use of this strategy for the construction of multicomponent nanoscale materials through self-assembly.

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