DOI: 10.1017/S1431927605508079
Microsc Microanal 11(Suppl 2), 2005

Synthesis, Characterization and Bioimaging Applications of Multi-Functional Dendrimer-Based Probes

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Live optical imaging is an extremely valuable tool used to streamline biological processes, such as development and regeneration, and therefore determine dynamic parameters such as velocity of motion or/and stability of a structure on one individual at multiple time points [1]. We will show our continued efforts towards the reliable preparation of customized multifunctional probes that allow for the simultaneous visualization using different microscopy modes.

The first generation of multi-functional probes was prepared by the conjugation of mono–sulfo-NHS-Nanogold® TDA with 10 KDa molecular weight amino-functionalized dextran conjugated to Texas Red. Such probes have been used as retrograde neuronal tracer in female and male Western Mosquito fish [2]. Even though the preliminary results were promising, the use of a polymeric core (aminodextran) resulted in poor batch-to-batch reproducibility.

Most limitations encountered by using polymeric-based multi-functional probes can be overcome by using dendrimers. Dendrimers are large and complex and usually monodispersed "star shaped" polymers with well-defined chemical structures [3]. They consist of three major architectural components: core, branches, and active sites or end groups and are produced by a sequence of reactions after which each step leads to the formation of a new layer and by consequence a new generation dendrimer. Therefore, one of the most appealing aspects of dendrimer-based technologies is the relative ease by which one can control their size, composition and chemical reactivity [3].

The applications for dendrimers have expanded in many diverse fields such as: drug delivery, diagnostics, catalysis, photo and electro active materials, DNA micro arrays, etc [4]. The structure of dendrimers makes them very good candidates for the precise construction of well-defined multi-functional probes, an aspect of dendrimer-based technology that has no been exploited so far.

Our prototype is based on a poly-lysine peptide being derivatized at the ε-NH_2 in the side chain of a Lys with a functionalized carboxylic acid. The general design of the probes has the following characteristics: modular [5] and iterative solid support synthesis (Figure 1); systematic variation of properties; customizable; biocompatible; quantitative and dynamic.

The synthesis of the dendritic domains (dendronized acids) has been performed as shown in scheme 1. Protection of the terminal hydroxyl groups with *p*-methoxy benzyl moieties (PMB-OH) due to their facile removal and compatibility with Fmoc peptide synthesis. Iterative double substitution reactions on the epichlorohydrin, by PMB-ONa or a lower generation dendron, provide the basic subunit (**A**) that can in turn be further reacted with epichlorohydrin (to make a higher-generation dendron (**B**)) or alkylated with ethyl bromoacetate followed by hydrolysis, to obtain the desired dendronized acid (**C**).

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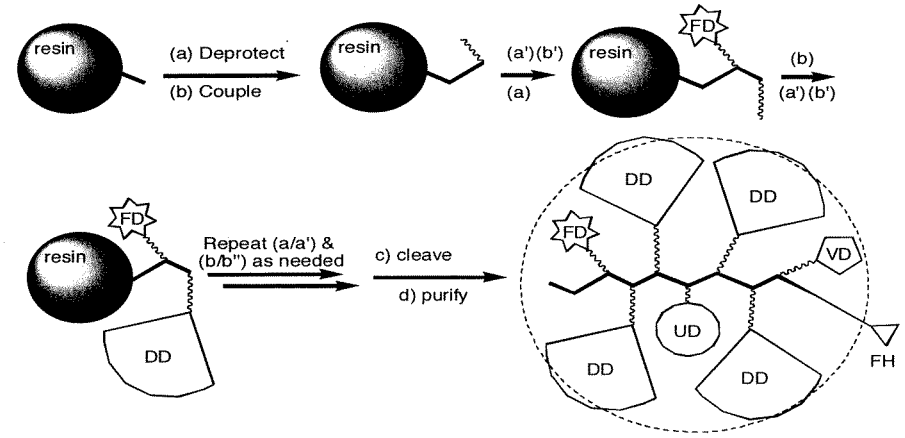
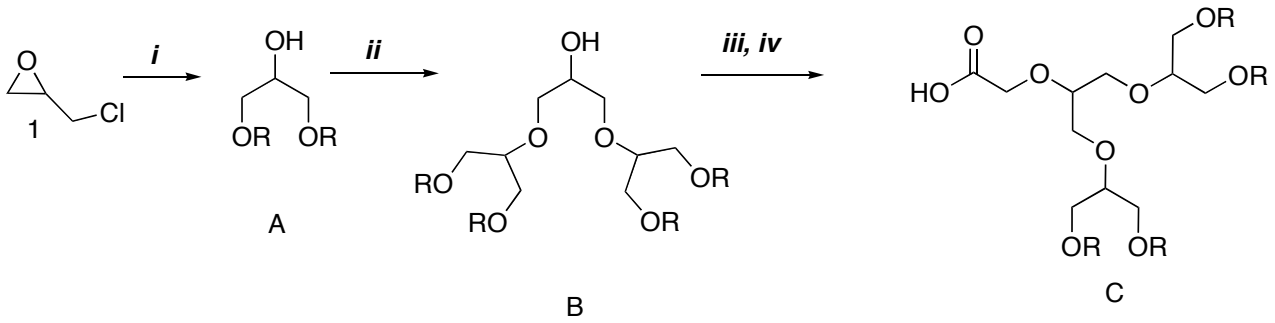
Figure 1



Schematic representation of the multifunctional probes synthesis based on standard Fmoc solid phase synthesis.
Key: Dendritic Domain (DD); Fluorescent Domain (FD); Ultrastructural Domain(UD); Variable Domain(VD) ; Fixation Handle (FH).

Scheme 1



R = PMB

i = PMB-ONa, THF, 65°C
ii = NaH, **1**, THF, 65°C
iii = Ethyl bromo acetate, NaH, THF, 0°C
vi = NaOH, THF/H_2O

https://doi.org/10.1017/S1431927605508079 Published online by Cambridge University Press