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High Loaded Dendrimers with Free Peripheral Groups

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ABSTRACT

We report the synthesis of dendrons and dendrimers with branching units composed of two parts: a residue with biological activity and a branching molecule. This approach allows better exploitation of the dendritic architecture, giving to the scaffolding structure an 'active' role beyond the traditional one and leaving the peripheral groups unaffected and ready for diverse modifications in order to improve their potential performance in the drug delivery scenario.

The dendritic architecture has been exploited as a versatile platform since its origins owing to its very controllable structure, low polydispersity, interior voids, and geometric growth of terminal functional groups.

Basically, there are two strategies for dendrimer synthesis, which employ iterative reaction steps known as divergent and convergent leading to a macromolecule with radial growth from a central core with characteristic regular and symmetrical branches that create voids within them and an exponential growth of the terminal functional groups depending on the number of branching points and their multiplicity, where the term generation is defined by the number of radial branching points[1]. Those particular features give dendrimers a unique behavior in different environments that has been exploited for the research and development of diverse applications including light harvesting[2,3], energy transfer[4], nanocatalysis[5], diagnostics[6], transfection vectors[7] and controlled drug delivery[8].

In the field of drug delivery, a review[9] of the biocompatibility and toxicity of dendrimers show the strategies that have been employed to take advantage of their topology in nanodevices for medical applications which can be grouped in three main groups: the use of the core, the "dendritic box", and the multiple terminal functional groups. The first one employs a functional entity as a core for the dendrimer growing[10]. In the dendritic box, the natural internal voids formed as the dendrimer generations grow can be occupied with host small molecules through non-covalent interactions[11, 12]. In the latter, it is possible to attach as many molecules as functional groups are in the periphery or the dendrimer. There are multiple examples and reviews in the literature on this, including non-covalent and covalent attachment of the drug to the dendrimer's peripheral structure[13-15]. Nowadays, in its most advanced form of a multifunctional drug delivery device, it is necessary to have high generation dendrimers to construct a molecular device with several features like active payload, targeting, tagging and solubilizing to meet desirable pharmacokinetic and pharmacodynamics properties, because each improvement in one of those properties is in detriment of the others and relies heavily on a statistical average of payload[16].

Furthermore, in the traditional synthetic strategies little attention has been paid to the scaffolding building blocks of the dendrimers and its exploitation as a strategic element beyond its branching function. Considering this, we focus on this concept to approach a more versatile version by considering the conventional branching molecule structured by a two-part element, the active load and the branching element coupled together by a suitable functional group. The introduction of active load directly bonded to the nucleus of every dendrimer generation permits perfect control on them and avoids the incomplete attachment commonly found with high generation dendrimers leaving the peripheral functional groups free for modifications pursuing properties tune-up. An example of this extension is the divergent synthesis of dendritic prodrug or "drug tree" dendrimers consisting in molecules of salicylic acid and L-Dopa as branching and active molecule simultaneously[17].

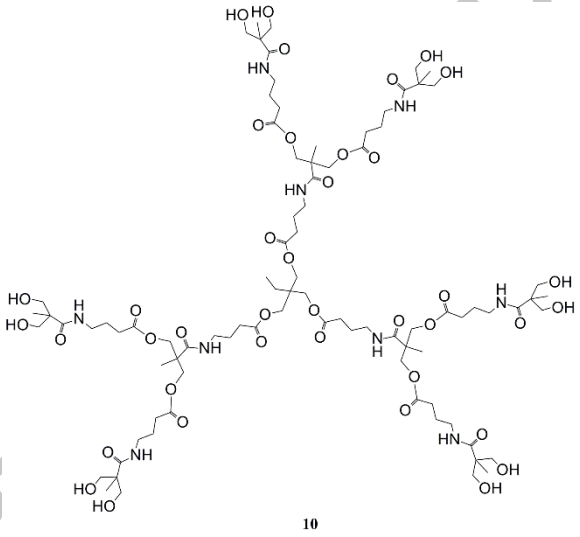
The maximum number of active load, Z, that can be achieved by this approach equals the number of covalent bonds forming the scaffold present in the particular dendrimer plus the number of peripheral functional groups and can be calculated by equation 1, where N_c is the

number of functional groups in the core molecule, N_b the number of functional groups of the branching element and G the number of generations of the dendrimer.

$$Z = N_c \sum_{i=0}^{G} N_b^i \qquad \text{eq. 1}$$

For a third generation dendrimer with a trifunctional core and a bifunctional branching molecule is possible to have 21 active molecules as a part of the scaffolding structure of the dendrimer and still have the 24 peripheral functional groups free for further key modifications, which is equivalent to 88% of the terminal groups. This is equivalent to a fourth generation conventional dendrimer with 48 end groups. The advantages of this approach is the exact control on the active payload, the total availability of the peripheral group and the use of lower generation dendrimers, a very desirable feature to scaling the dendrimer synthesis for mass production which is a current disadvantage. Obviously a requisite for this type of dendrimers is their degradability in the final media by non-specific enzymatic or chemical action which can be accomplished and modulated by selecting suitable functional groups as esters or amides, for instance.

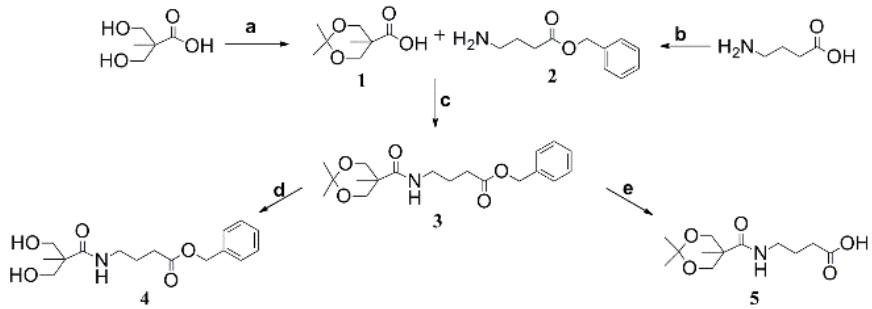
The divergent synthesis allows to build dendrimers with uniform load through the structure or homogeneous generational diversity[18], but the convergent strategy is the most versatile in to take advantage of this approach making possible the incorporation of branch and generational active load diversity in several ways leading to a precisely controlled "active cocktail" useful for fine modulation or multiple biological responses at multiple target active sites for synergistic effect. In order to achieve this, a biodegradability in its final destiny is a requisite imposed for the dendrimer.



10

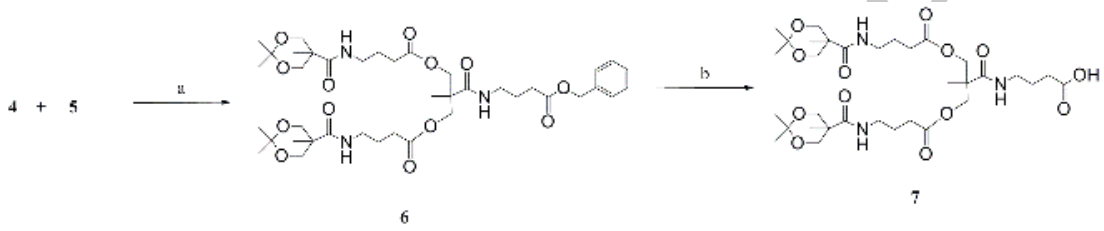
Figure 1. Second generation dendrimer with GABA residues in its structure.

Here we report for the first time the divergent and convergent synthesis of first and second generation dendrons and dendrimers based on gamma-aminobutyric acid (GABA) as an active load. GABA has been identified as the main inhibitory neurotransmitter in the central nervous system (CNS) and it is implied in several diseases as epilepsy, Parkinson and Alzheimer diseases. GABA is a polar molecule and is not a therapeutic alternative because its impossibility to cross the blood-brain barrier and a suitable carrier for its delivery would be very useful. The nine GABA molecules can be seen as an integral part of the dendrimer's structure (Fig. 1) linked by amide and ester functional groups, both of them susceptible of chemical or enzymatic hydrolysis. The twelve terminal hydroxyl functional groups are useful for covalent link of moieties for improving PKPD properties.



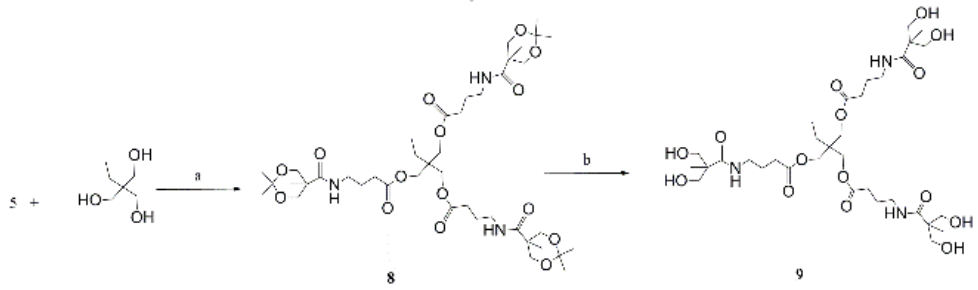
Scheme 1. Reagents and conditions: (a) dimethoxypropane, acetone, TsOH, 92 %; (b) benzyl alcohol, toluene, TsOH, 90%; (c) DCM, TsOH, DCC, rt, 68%; (d) 80% AcOH, rt, 98%; (e) MeOH, Pd(C), H_2 (40 psi), rt, 98 %.

The synthesis of the GABA loaded dendrimer **10** was accomplished by means divergent and convergent strategies and the use of the nontoxic and biocompatible building blocks trimethylol propane as a core and 2,2-bis(hydroxymethyl)propanoic acid (bisMPA) as a branching unit. Initially the basic building blocks bisMPA and GABA are modified with orthogonal protecting groups, hydroxyl groups of bisMPA are converted to a ketal **1** with dimethoxypropane/TsOH and the carboxylic group of GABA is transformed to the corresponding benzyl ester **2** by Fischer esterification procedure using a little excess of p-toluenesulphonic acid over the GABA as neutralizing agent for the GABA amine group to avoid the amide formation and catalysis (Scheme 1). The ester was formed straightforward in 90% yield. These two compounds are reacted to obtain the main building block **3** by means of an amide linkage using dicyclohexyl carbodiimide (DCC) as a dehydrating agent and dimethyl amino pyridinium toluenesulphonate salt (DMAPTS) as a catalyst. In this reaction, the compound **2** is in its toluenesulphonate salt and was necessary to add dimethyl amino pyridine (DMAP) in equivalent amount to form the DMAPTS in situ to avoid the prior benzyl ester purification step to remove the p-toluenesulphonic acid used in the Fischer esterification reaction. Other methods to get available the amino group, like alkaline treatment at a pH higher than the amino group pK, were unsuccessful. Despite the large amount of DMAP used, the reaction results in a simple to purify reaction mixture by successive extractions with 10% aqueous citric acid, 10% aqueous sodium bicarbonate, and deionized water followed by a column chromatography leading to a 68 % yield. The protecting groups in compound **3** are removed in separate steps to obtain the compounds **4** and **5**. Treatment of **3** with 80 % acetic acid for several hours was effective for the quantitative removal of the ketal protecting group with no ester hydrolysis.



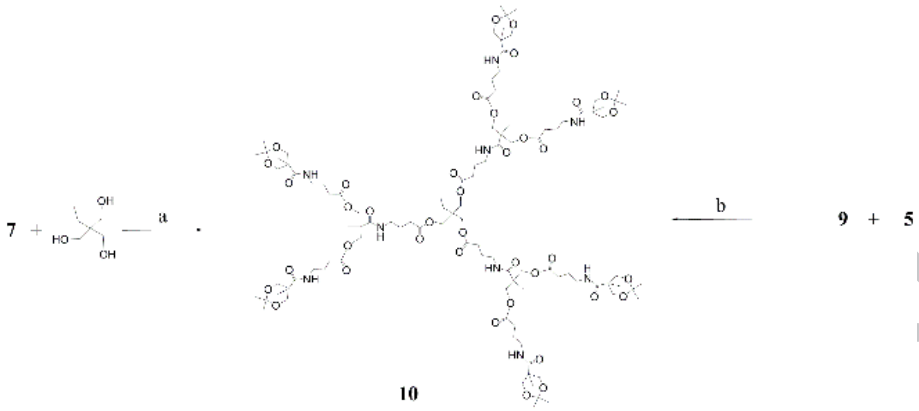
Scheme 2. Reagents and conditions: (a) DCM, DMAPTS, rt, 70%; (b) MeOH, Pd(C), H_2 (40 psi), rt, 97 %.

Treatment of **3** with a strongly acid ionic exchange resin as reported elsewhere was ineffective leading to byproducts formation and ester hydrolysis. The benzyl group deprotection to obtain **5** was quantitatively accomplished by low pressure (40 psi) hydrogenolysis with palladium on carbon catalyst, the debenzylation was 95% completed within the first hour.



Scheme 3. Reagents and conditions: (a) DCM, DMAPTS, 60%; (b) MeOH, Pd(C), H_2 (40 psi), rt, 98%.

Dendron **7** can be obtained reacting **4** and **5** using DCC/DMAPTS strategy with 70 % yield and then treated with low pressure hydrogenolysis with palladium on carbon as catalyst with quantitative yield (Scheme 2). The synthesis of the dendrimer of first generation **8** was accomplished reacting **5** with trimethylol propane and DCC/DMAPTS conditions with no loss of the ketal protecting groups and a 54 % yield. Dendrimer **8** showed solubility in dichloromethane. The quantitative conversion of dendrimer **8** into dendrimer **9** was done with 80 % acetic acid treatment. Dendrimer **9** with six hydroxyl groups is water soluble (Scheme 3).



Scheme 4. Reagents and conditions: (a) DCM, DCC, DMAPTS, 47 %; (b) DCM, DCC, DMAPTS, 60%.

The second generation dendrimer **10** was obtained by the convergent way (Scheme 4, reaction a) by condensing **7** with trimethylol propane using DCC/DMAPTS system again. The synthesis of **10** by divergent strategy can be done by reacting the first generation dendrimer **9** with equivalent relationship of **5** using the same condensation strategy (Scheme 2, reaction b). In both strategies the yields were in the order of 40-45%. The analysis showed the presence of incomplete coupling byproducts as a one and two molecules of **7** coupled to trimethylol propane nucleus before the work-up of the reaction mixture in the convergent synthesis while the divergent synthesis showed incomplete coupling of **5** to the hydroxyl groups of the first generation dendrimer **9**. The dendrimer **10** can be deprotected by an 80 % aqueous solution of acetic acid quantitatively to free the hydroxyl functionalities.

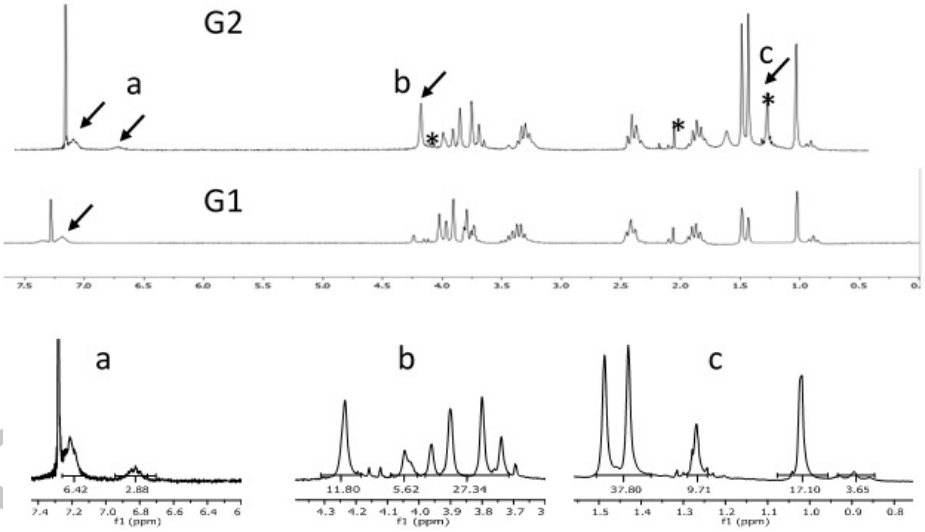


Figure 2. Comparison and details of GABA dendrimers of first (G1) and second generation (G2). Details of some of the second generation dendrimer are in the lower part of the figure. Arrows pointing to characteristic features of the G2 dendrimer structure (see text). The asterisks show residual solvent (ethyl acetate) used in the purification steps.

The structures of the dendrimers were fully characterized by NMR analysis (^{1}H and ^{13}C) and by HPLC-MS using ESI-TOF mass analysis. The NMR spectra signals and integration comparison between first and second generation dendrimers are consistent with the expected. The second generation spectra (Fig. 2, upper part, G2) shows the corresponding proton signals for the amide protons for the first and second generation portions in the 6.5-7.5 ppm region (Fig. 2, a) the decoupled methylene protons for the bisMPA of the first generation at 4.23 ppm and the coupled methylene protons of the bisMPA of the second generation (Fig. 2 b) and, finally, the new proton signal for the methyl groups of the first generation bisMPA methyl groups (Fig. 2, c) confirming the structure.

In conclusion, we synthesized dendrimers of first and second generations with a very controllable high load integrated in its scaffolding structure by convergent synthesis, which opens a new way of potential exploitation of the dendritic architecture in drug delivery applications. The focus is in covalently connecting an active molecule with a branching one to form a two part building block. The free peripheral groups can be modified by a convenient moiety like a solubilizer, targeting or tagging from the first step instead of a temporary protecting group, or

this can be done as a last step over the dendrimer. An interesting feature of the use of convergent strategy is that every building block is a prodrug by itself. This opens a new approach for drug delivery strategy.

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Supplementary data

Supplementary data (synthesis procedure and analytical data) associated with this article can be found in the online version.

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Graphical abstract

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