



Structural characterization of new defective molecules in poly(amidoamide) dendrimers by combining mass spectrometry and nuclear magnetic resonance



Aura Tintaru^a, Rémi Ungaro^a, Xiaoxuan Liu^b, Chao Chen^b, Laurent Giordano^c, Ling Peng^b, Laurence Charles^{a,*}

^a Aix-Marseille Université – CNRS, UMR 7273, Institut de Chimie Radicalaire, Marseille, France

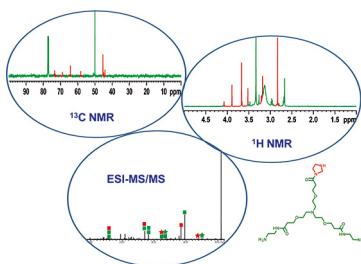
^b Aix-Marseille Université – CNRS, UMR 6114, Centre Interdisciplinaire de Nanosciences de Marseille, Marseille, France

^c Aix-Marseille Université – CNRS, UMR 7313, Institut des Sciences Moléculaires de Marseille ISM2 & Ecole Centrale de Marseille, Marseille, France

HIGHLIGHTS

- ESI-MS/MS and NMR were combined to elucidate a new side-reaction during divergent synthesis of PAMAM dendrimers.
- These new impurities exhibit a net gain of a single carbon atom as compared to expected molecules.
- The side-reaction is due to formaldehyde, contained as trace level impurity in methanol used as the synthesis medium.

GRAPHICAL ABSTRACT



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ABSTRACT

A new side-reaction occurring during divergent synthesis of PAMAM dendrimers (generations G₀–G₂) was revealed by mass spectrometric detection of defective molecules with a net gain of a single carbon atom as compared to expected compounds. Combining MS/MS experiments performed on different electro sprayed precursor ions (protonated molecules and lithiated adducts) with NMR analyses allowed the origin of these by-products to be elucidated. Modification of one ethylenediamine end-group of perfect dendrimers into a cyclic imidazolidine moiety was induced by formaldehyde present at trace level in the methanol solvent used as the synthesis medium. Dendrimers studied here were purposely constructed from a triethanolamine core to make them more flexible, as compared to NH₃- or ethylenediamine-core PAMAM, and hence improve their interaction with DNA. Occurrence of this side-reaction would be favored by the particular flexibility of the dendrimer branches.

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1. Introduction

Due to their numerous primary amine end-groups which participate in DNA binding processes, dendrimers such as poly

(amidoamine) (PAMAM) and poly(propyleneimine) (PPI) are promising non-viral gene delivery vectors [1–4]. Such properties could be used to bind to biologically important RNA molecules [5], opening the route to the use of dendrimers to target RNA as well as to deliver RNA agents. Interestingly, however, partially degraded PAMAM dendrimers, which offer more flexible structures as compared to perfect ones, were reported to interact more efficiently with DNA [6]. A successful strategy to increase flexibility

* Corresponding author. Tel.: +33 491 28 8678; fax: +33 491 28 2897.

E-mail address: laurence.charles@univ-amu.fr (L. Charles).

of dendritic molecules, while maintaining a high degree of control on their structure, consists of using a large moiety as the core. In this framework, the use of triethanolamine as the initiator in the conventional divergent approach for synthesizing PAMAM [7] allowed the production of dendrimers with branching units starting away from the center amine with a distance of ten successive bonds (Scheme 1), hence exhibiting less densely packed branching units and end-groups than the commercially available PAMAM dendrimers produced from NH_3 or ethylenediamine core [8].

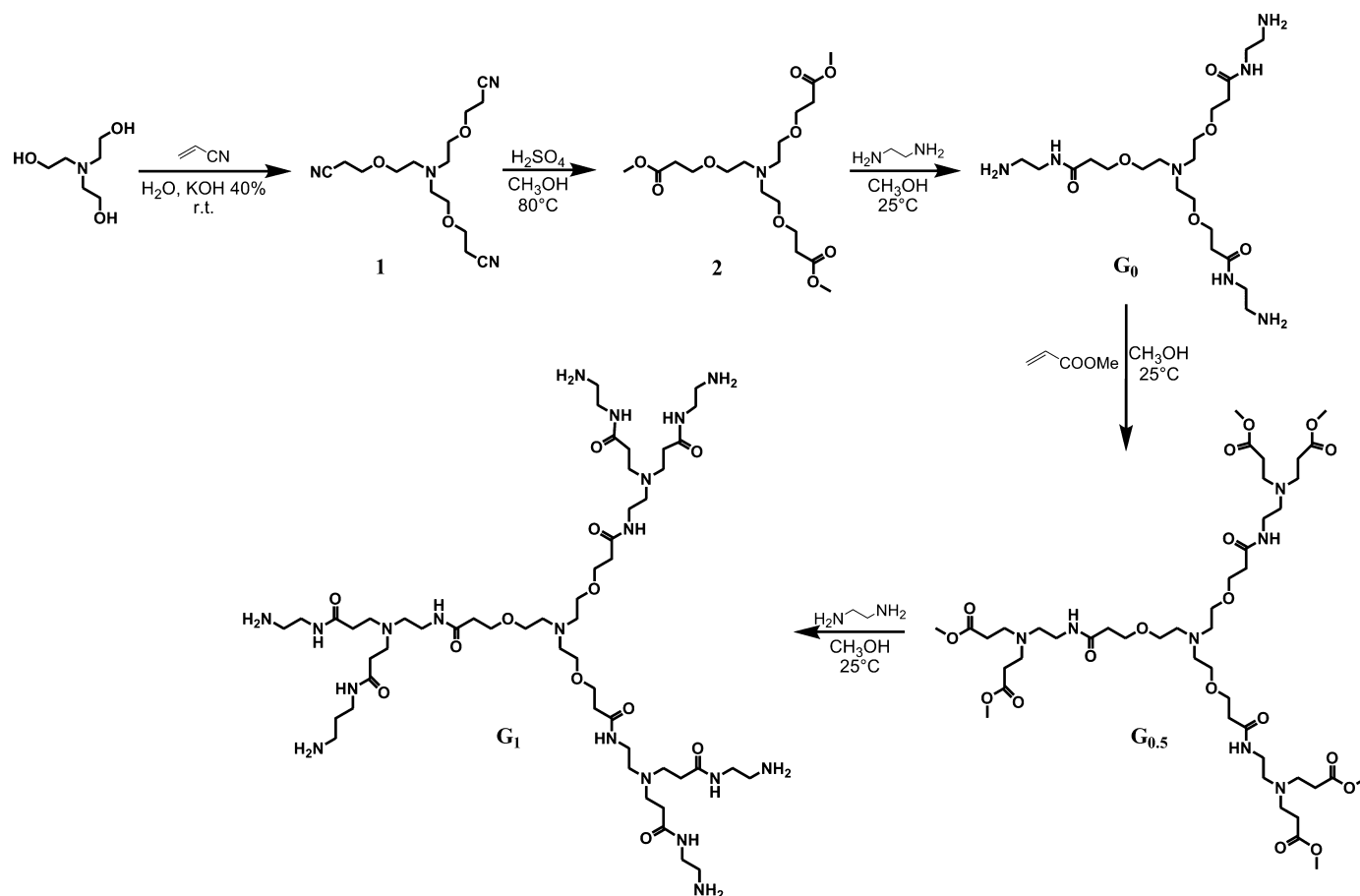
The so-called divergent approach used to prepare such triethanolamine-core PAMAM is very efficient for the production of very high generation dendrimers but is also known to generate a number of statistical defects. This synthesis methodology is based on a multi-step iterative reaction sequence, consisting of Michael addition of methyl acrylate to an amine-terminated core, followed by the amidation of the resulting methyl ester derivatives with diethylamine to regenerate the terminal amines, and produces concentric shells of branches around the central core. Three main types of side reactions have been shown to occur during the production of PAMAM dendrimers [7], conducting to defective molecules whose structure can readily be established using mass spectrometric techniques [9–28]: the “missing arm” defect (resulting either from subquantitative success of the Michael addition or from the retro-Michael reactions), the “molecular loop” (due to intramolecular cyclization which may occur during the amidation step as ethylene diamine is a bifunctional reactant), and the “dimer” (which may form during the amidation step). While analyzing triethanolamine-core PAMAM (G_0 – G_2 generation) samples by electrospray ionization mass spectrometry (ESI-MS) to

investigate any influence of their flexibility on the production of defective molecules, these three typical defects were detected (*vide infra*), but only with very low abundance. In contrast, a major impurity was observed at $+12 m/z$ compared to ions assigned to the perfect dendrimers, regardless of the PAMAM generation. Such an ionic species present at $+12 m/z$ compared to ions expected from the targeted molecule was previously reported by Maire and Lange [29] in the case of an ethylenediamine-core PAMAM dendrimer. However, since these authors observed this impurity in the negative ion mode only, they concluded that its formation resulted from a reaction between one NH_2 dendrimer end-group and a decomposition product of methanol formed upon electrical discharges occurring in negative mode electrospray. In our case, however, detection of a peak at $+12 m/z$ compared to protonated molecules in the positive ion mode was clearly in favor of a defective dendrimer present in the electrosprayed solution. A combined analytical approach based on tandem mass spectrometry and nuclear magnetic resonance was then implemented to characterize this new defective molecule, in order to identify its origin.

2. Materials and methods

2.1. Chemicals

Methanol was from SDS (Peypin, France), formaldehyde, formic acid, ethylenediamine and lithium chloride were from Sigma-Aldrich (St. Louis, MO, USA). Deuterated chloroform (CDCl_3 , 99.8%) was from Euriso-Top (Saint-Aubin, France). All chemicals were used as received without further purification.



Scheme 1. Synthesis of the triethanolamine core PAMAM dendrimer.

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