By connecting a synthetic scaffold and a natural shell of gallic acid: an innovative double-acting antioxidant device to fight «oxidative stress»

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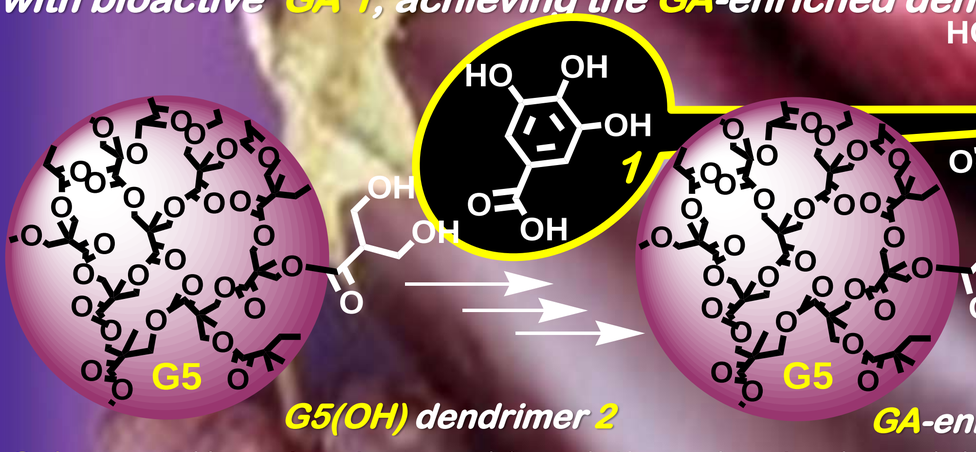
Background. Oxidative stress (OS) is the main triggering factor for the onset and developing of most degenerative diseases hard to fight with existing synthetic drugs without adverse side effects. Gallic acid (GA) **1**, is a natural triphenolic acid present in several plants, fruits and common foodstuffs (Fig.1). It is provided both with the basic nutritional values and with several extra health benefits such as a remarkable antioxidant power and it has exhibited abilities in protecting cells from OS via a number of pathways without triggering unpleasant side effects.[1] Unfortunately, GA clinical application is limited by its pharmacokinetic drawbacks, poor bioavailability, slow GIT absorption, fast metabolism and short half-life. Dendrimer nanoparticles, thanks to their nonpareil physicochemical properties, are extensively exploited in nanomedicine to control molecular weight, hydrophilicity, solubility,[2-4] bioavailability and pharmacokinetic behaviour of drugs as well as to protect them from early degradation or fast metabolism. Among dendrimers, PAMAMs are very efficient and continuously under study, but because of their non-biodegradability and excessive cationic charge, they may cause irreparable damage to cells and require laborious modifications to be in vivo used. Therefore, nowadays, uncharged polyester-based hydrolysable dendrimer scaffolds are preferred.

Present Work. With the aim at minimizing GA limitations for allowing its clinical applications, in this study, the G5 polyester-based dendrimer **2** with 64 peripheral OH functions exploitable for further esterification, was prepared and subsequently, it was decorated with bioactive GA **1**, achieving the GA-enriched dendrimer **3** containing sixty-four peripheral GA units (Scheme 1).



Scheme 1. Key structures achieved: the polyester-based dendrimer matrix **2** and the final GA-enriched dendrimer**3**

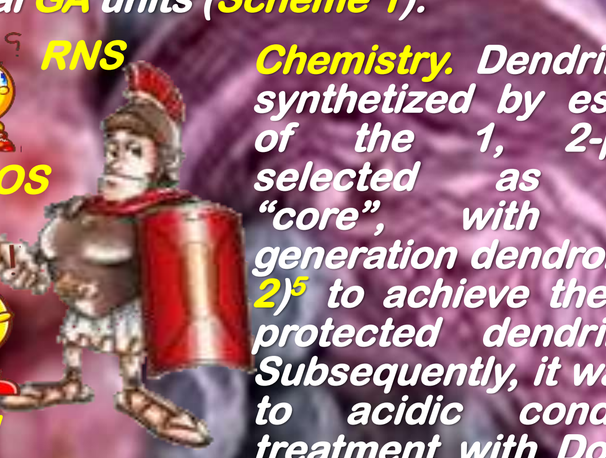
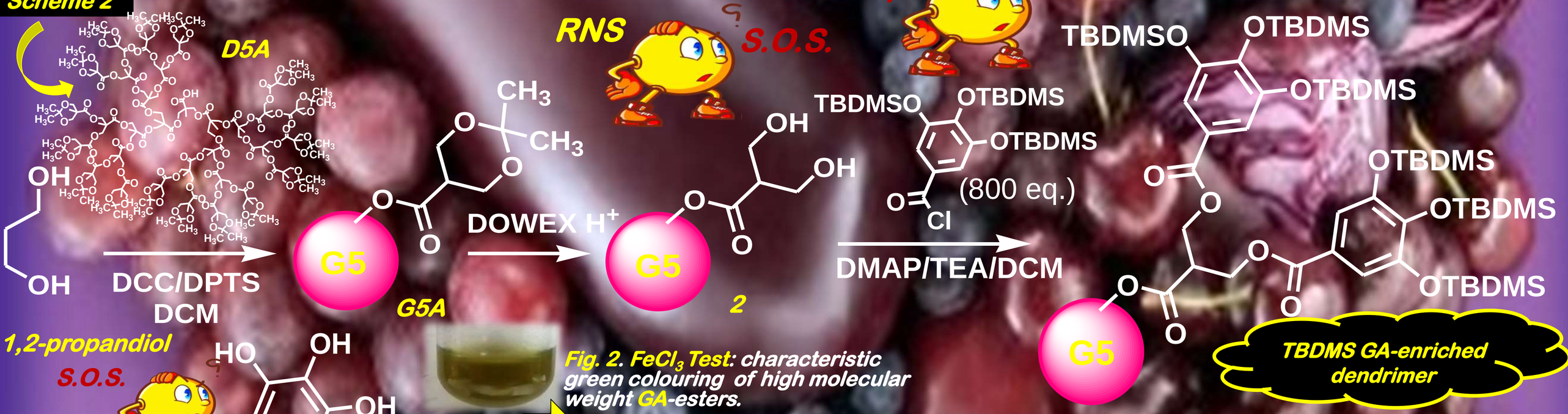


Fig. 1 Gallic acid **1**

Chemistry. Dendrimer **2** was synthetized by esterification of the 1, 2-propandiol, selected as dendrimer "core", with the fifth generation dendron D5A (Fig. 2)[5] to achieve the acetonide protected dendrimer G5A. Subsequently, it was exposed to acidic conditions by treatment with Dowex resins H^+ to give **2** (Scheme 2).

Scheme 2



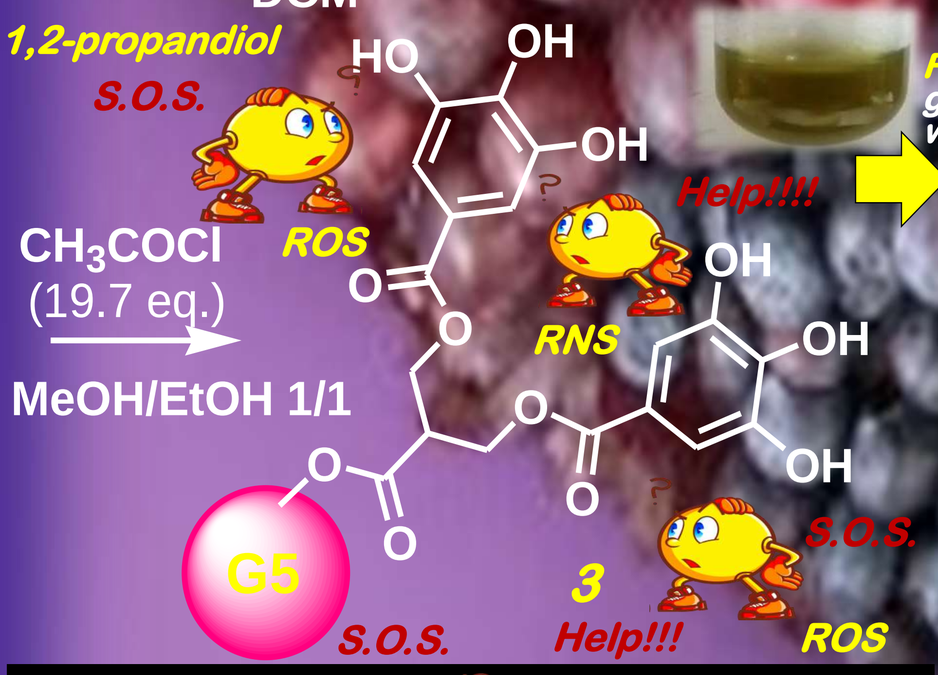
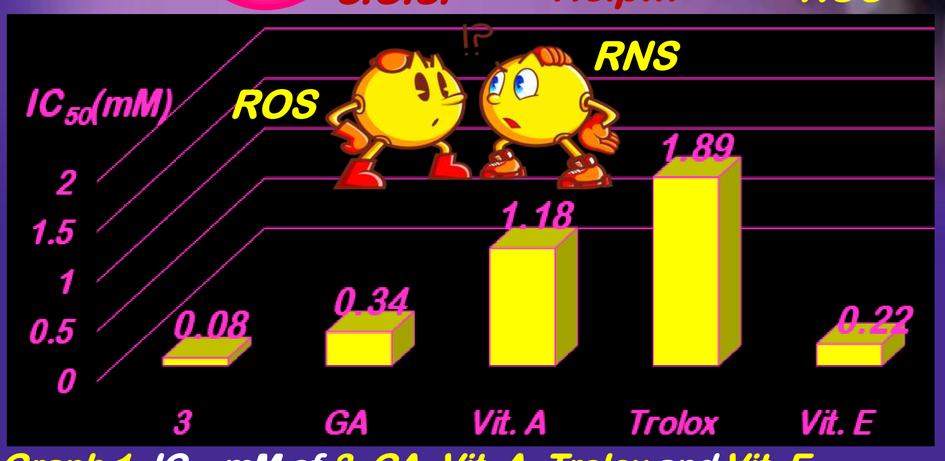


Fig. 2. $FeCl_3$ Test: characteristic green colouring of high molecular weight GA-esters.

Dendrimer **2** was esterified with the acid chloride derivative of **1** protected to the phenols groups[6] to avoid side reactions and/or uncontrolled polymerisation.The subsequent protecting groups removal, by the treatment with acethyl chloride and alcohols, provided the final GA-enriched dendrimer **3** (Scheme 2). Once the presence of free OH groups was proved by performing the $FeCl_3$ essay (Fig. 2), the structure of **3** was confirmed by FTIR, NMR and Elemental analysis. Then, **3** was investigated to determine its potentials as innovative antioxidant device to be used as safer alternative to common synthetic non dendrimeric drugs against OS.

Antioxidant activity and biodegradability investigations. Firstly, the intrinsic antioxidant power of **3** was determined by performing the DPPH test obtaining its Radical Scavenging Activity (RSA%). In parallel, for comparison, GA, Vitamin A, E and Trolox were also essayed. The results were reported as IC_{50} and are shown in Graph 1. Secondly, an in vitro reproduction of what could happen to **3** because of an enzymatic attack by cells esterase, was conceived and realized, for ascertaining the actual behaviour and fate of **3** once inside the cell. So, **3** was treated at 37 °C, for 24 hours, in PBS with the commercial Pig Liver Esterase (PLE). After the recover of the crude solid product, investigation by $FeCl_3$ assay, TLC analysis, recrystallization, m.p. and NMR, showed that the main detectable component was the bioactive GA.

Results. Dendrimer **3** showed an antioxidant power four time higher than free GA and proved to be able to degrade by cell esterase hydrolytic action to non cytotoxic small molecules setting free the bioactive GA units for additional antioxidant effects, as depicted in Fig. 3.



Graph 1. IC_{50} mM of **3**, GA, Vit. A, Trolox and Vit. E

CONCLUSIONS. By merging synthetic processes and the healthy chemistry of natural compounds, this study led to get a GA-enriched biodegradable, non cytotoxic antioxidant dendrimer (**3**) more powerful than free GA and than other known antioxidants. Compound **3** also embodies the features of a GA delivering device, able to carry several bioactive GA units at once, thus improving its pharmacokinetic and preserving it from fast metabolism. In physiological condition, i.e. as inside the cell, **3** degrades to non cytotoxic molecules and releases bioactive GA units by esterase action. The achieved GA-prodrug can be considered an innovative double-acting therapeutic with high potentials for the treatment of diseases triggered by OS.

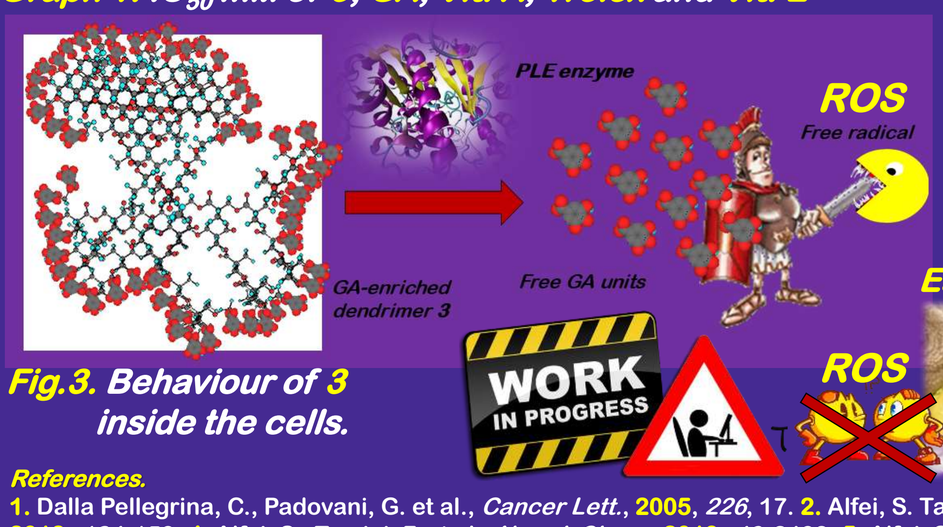


Fig.3. Behaviour of **3** inside the cells.

Studies currently in progress, are proving that dendrimer **3** works very well and much more better than the free GA as a preservative additive against the oxidative degradation of essential oils, as an inhibitor of platelet aggregation and as an antibacterial.

References.
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