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# Efficient modification of PAMAM G1 dendrimer surface with $\beta$ -cyclodextrin units by CuAAC: impact on the water solubility and cytotoxicity†

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The toxicity of the poly(amidoamine) dendrimers (PAMAM) caused by the peripheral amino groups has been a limitation for their use as drug carriers in clinical applications. In this work, we completely modified the periphery of PAMAM dendrimer generation 1 (PAMAM G1) with  $\beta$ -cyclodextrin ( $\beta$ -CD) units through the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) to obtain the PAMAM G1- $\beta$ -CD dendrimer with high yield. The PAMAM G1- $\beta$ -CD was characterized by <sup>1</sup>H- and <sup>13</sup>C-NMR and mass spectrometry studies. Moreover, the PAMAM G1- $\beta$ -CD dendrimer showed remarkably higher water solubility than native  $\beta$ -CD. Finally, we studied the toxicity of PAMAM G1- $\beta$ -CD dendrimer in four different cell lines, human breast cancer cells (MCF-7 and MDA-MB-231), human cervical adenocarcinoma cancer cells (HeLa) and pig kidney epithelial cells (LLC-PK1). The PAMAM G1- $\beta$ -CD dendrimer did not present any cytotoxicity in cell lines tested which shows the potentiality of this new class of dendrimers.

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## Introduction

In the last decades, research on the development of new dendrimers has become more relevant and attracted the attention of the scientific community, since these compounds have unique properties that make them ideal carriers for therapeutic and diagnostic agents in the construction of drug delivery systems (DDS).<sup>1</sup> One advantage of dendrimers is that between the small molecules and the polymers, dendrimers benefit from the properties of both fields. Given the versatility to modify them (like polymers), it is possible to guarantee a perfectly defined and reproducible structure.<sup>2</sup> It has been described that the groups in the periphery are largely responsible for the physicochemical properties of dendrimers and can be modified in a controlled manner to yield monodisperse materials.<sup>3</sup>

One of the most studied dendrimers, is the family of poly(amidoamines) (PAMAM), which is constituted by a core of

ethylenediamine or ammonia, repetitive units of polyamides, tertiary amines and a periphery containing primary amines.<sup>4</sup> It has been found that low generations (G 0–3) of PAMAM dendrimers tend to form open and amorphous structures, and therefore do not exhibit well defined internal characteristics, while high generations dendrimers (G 4–10) adopt spherical and rigid structures that allow them to encapsulate drug molecules in their branches.<sup>5,6</sup>

At physiological conditions (pH = 7.4) the primary amines of the PAMAM dendrimers are positively charged,<sup>7</sup> they exhibit toxicity, since they interact with the cell membrane, which is negatively charged, compromising its integrity and filtering intracellular components, that finally lead to a cell death. This toxicity is also associated with the increase in generation, due to the augment in the number of terminal amino groups. For this reason, the clinical application of PAMAM dendrimers is quite limited.<sup>8,9</sup>

In recent years, research has focused on improving the biocompatibility of PAMAM dendrimers by surface engineering, that means the modification of the periphery to neutralize the primary amines that are positively charged on the surface, thereby preserving the ability to deliver drugs. There are several strategies to modify the periphery of PAMAM dendrimers, such as coupling with carbohydrates, amino acids or peptides, acetylations and PEGylations.<sup>8</sup> One of the strategies used in surface engineering of PAMAM dendrimers is the coupling with cyclodextrins because it increases the solubility, stability and biocompatibility of dendrimers and therefore causes a synergistic improvement in the administration of drugs.<sup>10</sup>

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Chemically, cyclodextrins (CDs) are cyclic oligosaccharides linked through  $\alpha$ -1,4 glucosidic bonds. The nomenclature of the CDs is based on the number of glucose units contained in their structure, so that the CDs containing six units are called alpha-CD ( $\alpha$ -CD), seven units, beta-CD ( $\beta$ -CD) and eight units, gamma-CD ( $\gamma$ -CD).<sup>11</sup> As a result of the restricted rotation of the bonds in the glucose units by the chair conformation, CDs molecules have a truncated cone shape with a cavity of determined volume. The hydroxyl groups attached to the carbons 3 and 4 are located on the secondary face, and the hydroxyl groups attached to carbon 6 are located on the primary face of the CDs; these hydroxyl groups have a different reactivity, because they are attached to secondary and primary carbons, respectively. The surface of the CDs is coated with hydroxyl groups and the inner part is coated with ether groups of anomeric oxygen atoms. Therefore, CDs have a relatively hydrophobic internal cavity, which contains water molecules and a hydrophilic outer surface. This special conformation allows the inclusion of hydrophobic molecules or parts of molecules within the CD cavity, which must have a compatible size with the cavity of CD, to form a stable inclusion complex.<sup>12</sup>  $\beta$ -CD is the most accessible and widely used CD in the design of DDS, due to the adequate size of its cavity to form inclusion complexes with drug molecules.<sup>13</sup>

On the other hand, the 1,3-dipolar cycloaddition reaction between an azide and a terminal alkyne catalyzed by copper(I) (CuAAC) which produces 1,4-disubstituted 1,2,3-triazoles was reported independently in 2001 by Meldal *et al.*<sup>14</sup> and Sharpless *et al.*<sup>15,16</sup> Meldal used copper iodide and *N,N*-diisopropylethylamine for the synthesis of peptides, while Sharpless described the use of copper sulfate pentahydrate and sodium ascorbate in aqueous systems and designed a practical procedure for the covalent attachment between any molecule containing an azide group and another molecule containing a terminal alkyne group. The reaction of CuAAC is modular, specific and regioselective, inert byproducts are obtained and offers high yields. In addition, this reaction is carried out under simple conditions and with accessible raw materials.<sup>17</sup> During the last decade, the CuAAC reaction has proven to be a reliable and versatile technique for the preparation of new functional materials,<sup>18,19</sup> including dendrimers as well as the modification of their periphery.<sup>3,20–24</sup>

There are several works that describe the conjugation of PAMAM dendrimers (G 2–4) with different CD molecules.<sup>25–32</sup> The incorporation from one to five CD units is carried out in the periphery of dendrimers with applications in the delivery of genetic material for the specific treatment of diseases. On the other hand, since low generations of PAMAM dendrimers do not show encapsulating properties of molecules, and particularly  $\beta$ -CD molecules, have a limited water solubility. Therefore, the clinical applications of low generations of PAMAM and of  $\beta$ -CD as single molecules are limited. Based on the previously mentioned properties, we present a novel design and synthesis of a PAMAM G1 conjugate with  $\beta$ -CD units throughout the periphery of dendrimer. This conjugation was achieved by means of a CuAAC reaction with high yields and a simple purification process. Furthermore, we focused on the study of

the *in vitro* cellular behavior of this novel dendrimer as part of the rational evaluation, since our perspective is based on the potential use of this PAMAM G1- $\beta$ -CD conjugate as a possible carrier of diverse molecules. The advantage of this new modified dendrimer is that it is able to carry a higher number of drug units than a single carrier.

## Results and discussion

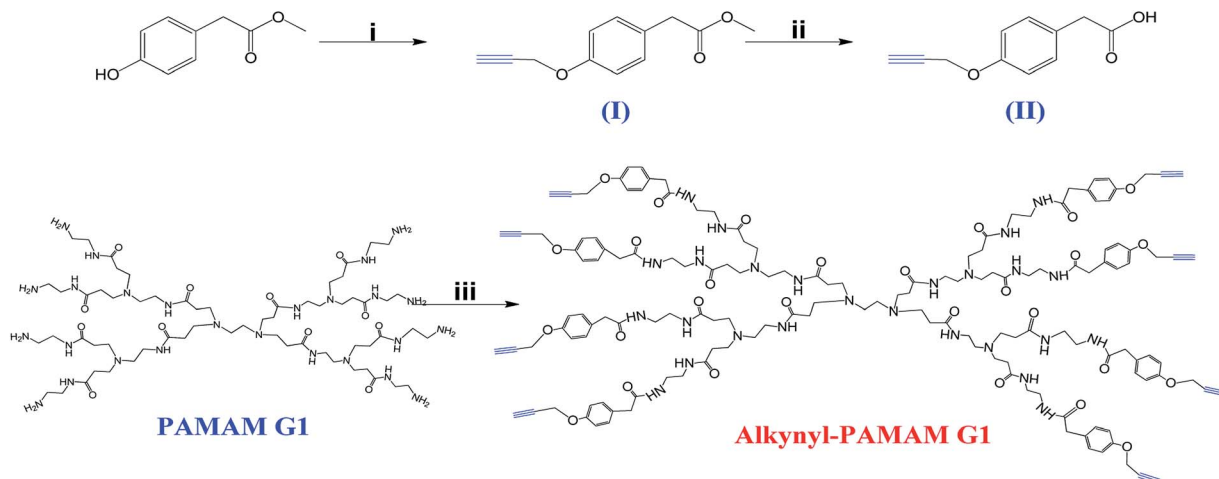
### Synthesis

In recent years, numerous reports described the advantages of PAMAM dendrimers in various areas of science and technology; however, there are also some disadvantages that limit their use in clinical applications. Firstly, PAMAM dendrimers are toxic and this toxicity can be only resolved by modifying the structure of the periphery of the dendrimer.<sup>33</sup> Intense research has focused on the surface engineering of PAMAM dendrimers with the aim of reducing or eliminating their toxicity by neutralizing the amino groups in the periphery by means of coupling of neutral or anionic groups in order to prevent the electrostatic interaction of PAMAM dendrimers with biological membranes to help them to be biocompatible for their use as drug carriers.<sup>8</sup> A wide variety of methods have been reported to modify the surface of PAMAM dendrimers, including modification with carbohydrates, specifically with oligosaccharides such as CDs, since it has been reported that a synergy of the properties of PAMAM dendrimers and CDs occurs.<sup>34–36</sup> PAMAM and  $\beta$ -CD dendrimers have been individually investigated as promising carriers for clinical applications; however, due to the toxicity of PAMAM dendrimers<sup>8,37</sup> and the low water solubility of  $\beta$ -CD,<sup>38</sup> their application in the clinical area is limited. In the present study we present a novel and efficient approach to modify the periphery of the PAMAM G1 dendrimer with  $\beta$ -CD units through the CuAAC reaction. All the intermediates and final compounds involved in the synthesis have been fully characterized.

Firstly, alkynyl-PAMAM G1 was synthesized (Scheme 1) and was used as alkyne source for the CuAAC reaction. A Williamson etherification reaction was carried out between propargyl bromide and methyl 2-(4-hydroxyphenyl)acetate, to obtain intermediate **I**, whose structure was confirmed by <sup>1</sup>H-NMR and <sup>13</sup>C-NMR and by ESI mass spectrometry (Fig. S1–S3 in the ESI†). Then, compound **I** was saponified *via* a simple hydrolysis with a yield >90% to obtain intermediate **II**. The structure of this compound was also confirmed by NMR and mass spectrometry (Fig. S4–S6 in ESI†).

Regarding the synthesis of the PAMAM G1-alkyne compound, we performed a C–N coupling between the amine groups present in the periphery of the PAMAM G1 dendrimer and the carboxylic acid of intermediate **II**, using HOBt and EDC as coupling agents and DIPEA as acid activator. These coupling agents are frequently employed for the preparation of amides<sup>39</sup> in the biomaterials field. It was reported that EDC is a very effective coupling agent for the synthesis of these compounds and when it is used jointly with HOBt, there is an increase in the efficiency of the reaction and the by-products can be easily removed in water.<sup>40,41</sup> The modification of the periphery of the PAMAM G1 dendrimer by introducing alkyne groups was





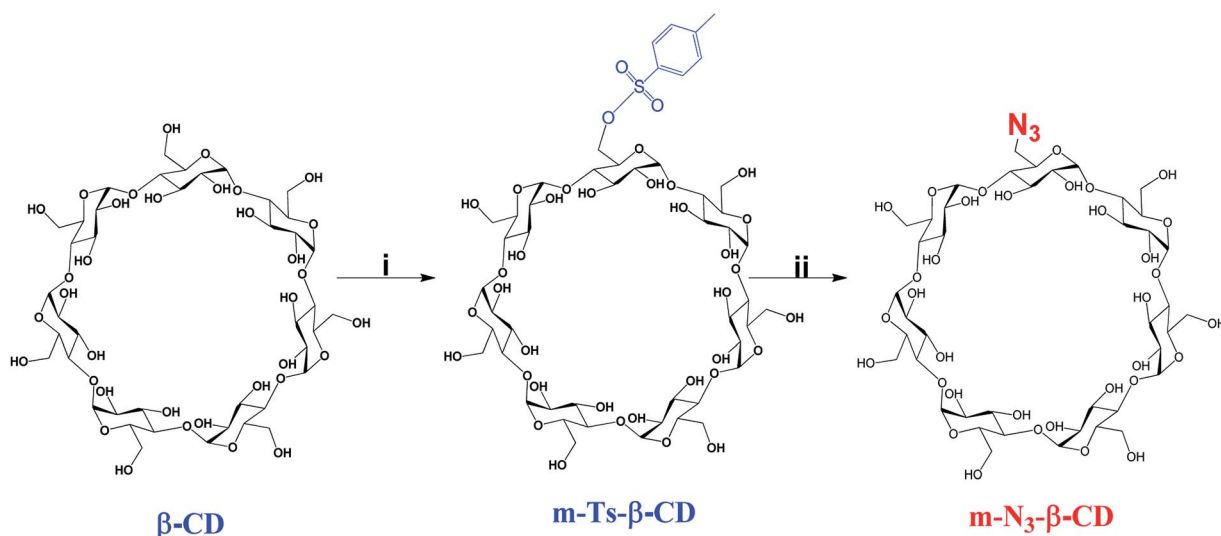
**Scheme 1** Synthesis of alkynyl-PAMAM G1. Conditions: (i) propargyl bromide,  $K_2CO_3$ , anhydrous acetone, 24 h, reflux; (ii) NaOH/HCl, EtOH :  $H_2O$ ; (iii) EDC, HOBt, DIPEA, DMF, 35 °C, 5 days.

confirmed by  $^1H$ - and  $^{13}C$ -NMR and by MALDI-TOF mass spectrometry (Fig. S7–S9 in ESI†).

On the other hand,  $m-N_3$ - $\beta$ -CD was synthesized as a source of azide for the CuAAC reaction (see Scheme 2). Firstly, the intermediary  $m-Ts$ - $\beta$ -CD was obtained by functionalizing a single hydroxyl group of the primary face of the  $\beta$ -CD *via* a tosylation reaction, which was further reacted with  $\beta$ -CD.<sup>42</sup> With this proposed method it was possible to modify a single position of the primary face of the  $\beta$ -CD with a high yield, compared to the previously reported procedures.<sup>43,44</sup> Tosylation of a single group of the  $\beta$ -CD was confirmed by  $^1H$ -NMR, DEPTQ-NMR and MALDI-TOF mass spectrometry (Fig. S10–S12 in ESI†). Then a bimolecular nucleophilic substitution reaction was carried out to replace the tosyl group of  $m-Ts$ - $\beta$ -CD with the azido group on the primary face of the  $\beta$ -CD to afford  $m-N_3$ - $\beta$ -CD, whose

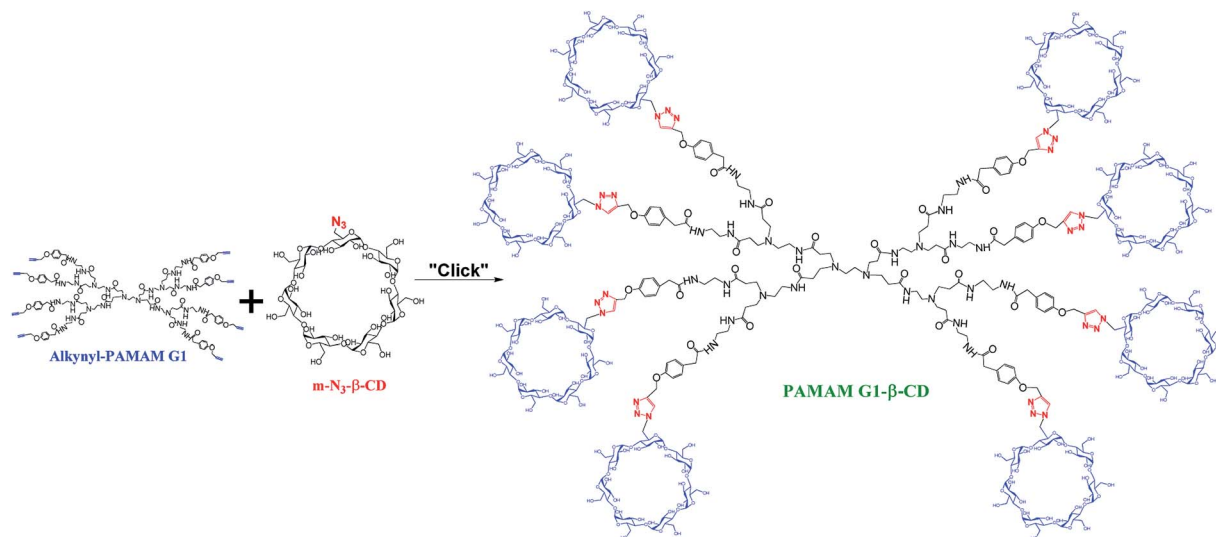
structure was confirmed by  $^1H$ -NMR, DEPTQ-NMR and mass spectrometry (Fig. S13–S15 in ESI†).

The CuAAC reaction allows a successful functionalization of biomaterials in a selective way<sup>45–53</sup> and has been often used to modify the surface of PAMAM dendrimers<sup>54–56</sup> because it offers high yields with a single reaction product obtained with great purity and harmless by-products easily removable.<sup>16,57</sup> In this work, we used the CuAAC reaction to efficiently functionalize the entire periphery of the PAMAM G1 dendrimer with  $\beta$ -CD units to build a novel dendritic structure, which can act as a potential drug carrier. Once the necessary intermediates for the reaction of CuAAC (Scheme 3), the alkyne (alkynyl-PAMAM G1) and the azide ( $m-N_3$ - $\beta$ -CD) were obtained, the coupling was carried out using  $CuSO_4 \cdot 5H_2O$  and  $H_2Asc$  to generate *in situ* Cu(I), which is the main catalyst in this reaction. To ensure the functionalization of the 8 alkyne groups of the modified



**Scheme 2** Synthesis of  $m-N_3$ - $\beta$ -CD. Conditions: (i)  $TsO_2/NaOH$ ,  $H_2O$ , 2 h, RT; (ii)  $NaN_3$ , DMF, 80 °C, 48 h.





Scheme 3 Synthesis of PAMAM G1- $\beta$ -CD. Conditions: "Click"  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{H}_2\text{Asc}$ , DMSO, 80 °C, 48 h.

periphery of the PAMAM G1 dendrimer, an excess of  $m\text{-N}_3\text{-}\beta\text{-CD}$  was employed. The PAMAM G1- $\beta\text{-CD}$  dendrimer was obtained with 98% yield and its structure was established by  $^1\text{H-NMR}$ , DEPTQ-NMR, elemental analysis by X-ray fluorescence (Fig. S16–S17 in ESI $^\dagger$ ). Dendrimer purification was performed by size exclusion chromatography (SEC) using water as eluent. This technique of purification proved to be highly efficient to remove the remaining copper and ascorbic acid, and thereby avoiding the classic method of dialysis.<sup>58</sup>

### Characterization

Full characterization of all the synthetic intermediates and final compounds was carried out. Regarding the characterization of intermediate **I**, in the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra, the signals corresponding to the alkyne group at 2.54 ppm and 75.54 ppm are observed, respectively. In addition, the disappearance of the signal corresponding to the proton of the phenol is observed. The molecular ion at 204.08  $m/z$ , corresponding to the molecular weight of the compound, is observed in the ESI spectrum. In the  $^1\text{H-NMR}$  spectrum of intermediate **II**, we can observe a signal at 12.25 ppm, due to the proton of the carboxylic acid, produced by the hydrolysis of intermediate **I**. The molecular ion is observed at 191.07  $m/z$  in the ESI spectrum, which corresponds to the molecular weight of this compound. On the other hand, in the  $^1\text{H-NMR}$  spectrum of alkyne-PAMAM G1, the signals corresponding to the alkyne group at 3.55 ppm are observed, and the proton signal of the 8 amide groups appear at 8.48 ppm, thereby confirming that the C–N coupling took place. In addition, the signals due to the protons of the aromatic ring appear at 7.18 and 6.90 ppm, exhibiting an up-field shift. Finally, the signals corresponding to the protons of the PAMAM G1 dendrimer are observed. In the MALDI-TOF spectrum, the molecular ion is observed at 2810.819  $m/z$ , which corresponds to the molecular weight of the multiprotonated species, which confirms the obtainment of the alkyne-PAMAM G1 compound.

On the other hand,  $m\text{-Ts-}\beta\text{-CD}$  was characterized by  $^1\text{H-NMR}$  spectroscopy, the signals corresponding to the aromatic ring protons at 7.75 and 7.44 ppm and the methyl protons at 2.42 ppm of the tosyl group can be observed. In the MALDI-TOF spectrum the appearance of the peak at 1311.591  $m/z$  corresponding to the expected molecular weight of the compound plus a sodium ion confirmed the mono-substitution of the primary face of the  $\beta\text{-CD}$ . After the modification of  $m\text{-Ts-}\beta\text{-CD}$ , compound  $m\text{-N}_3\text{-}\beta\text{-CD}$  was obtained. In the  $^1\text{H-NMR}$  spectrum, the disappearance of the signals corresponding to the protons of the aromatic ring and the methyl protons of the tosyl group confirms its replacement by the azide group. The signal at 1182.764  $m/z$  in the MALDI-TOF spectrometry corresponds to the molecular weight of the compound, plus a sodium ion.

Once the CuAAC reaction was achieved, the PAMAM G1- $\beta\text{-CD}$  dendrimer was obtained and further characterized. In the  $^1\text{H-NMR}$  spectrum in  $\text{DMSO-}d_6$  (Fig. 1), a signal due to the triazole group appears at 8.16 ppm, followed by the signals of the aromatic ring at 7.17 and 6.97 ppm. The signals corresponding to the alkyne-PAMAM G1 and the  $m\text{-N}_3\text{-}\beta\text{-CD}$  exhibited a clear change in the chemical environment due to the conjugation between both moieties. The PAMAM G1- $\beta\text{-CD}$  dendrimer was also characterized by MALDI-TOF, using the following conditions: 2,5-dihydroxybenzoic acid (DHB) in relation (2/5) sample/matrix; ditranol (DIT) in relation (2/5) sample/matrix; DHB and  $\text{AgNO}_3$  as a cationizer agent, in ratios (2/5/1) sample/matrix/cationizer; DHB dissolved in  $\text{H}_2\text{O/ACN/TFA}$  in ratios (60/40/0.1); and 2-acetylphloroglucinol (THAP) dissolved in methanol in ratios (1/1), (3/4), (1/9) sample/matrix. Only the signals corresponding to the matrices were observed in the range 1000 to 20 000  $m/z$ . Result of the foregoing, it was not possible to characterize PAMAM G1- $\beta\text{-CD}$  dendrimer by MALDI-TOF. It has been reported in the literature<sup>40,59,60</sup> that PAMAM dendrimers are difficult to ionize and to be detected by mass spectrometry. This dendrimer was therefore characterized by elemental analysis and X-ray fluorescence, where the following values were



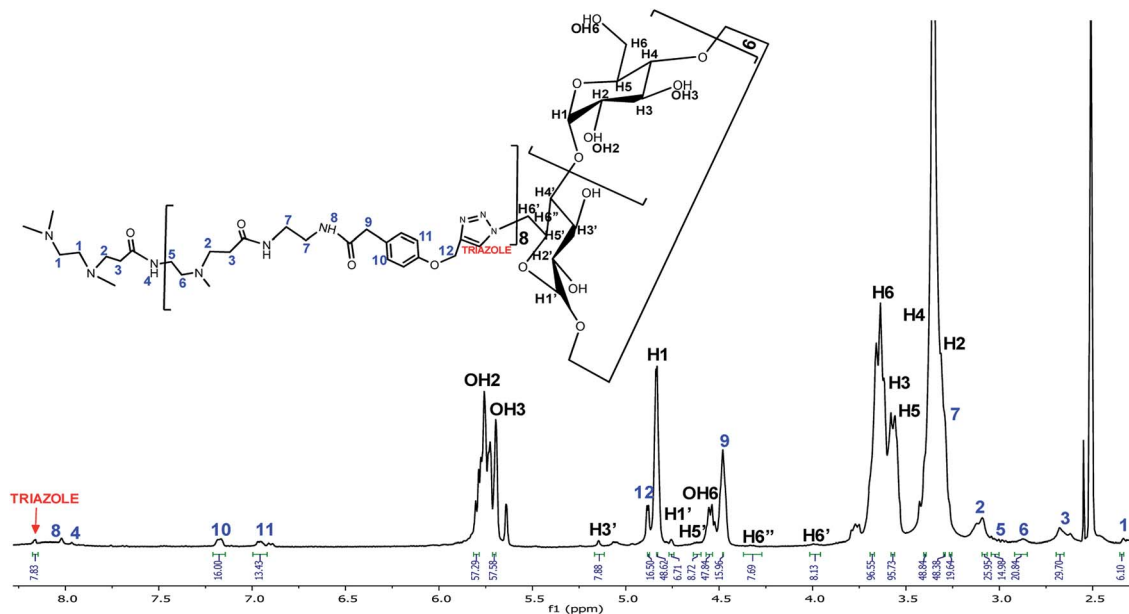


Fig. 1  $^1\text{H}$  NMR spectrum of PAMAM G1- $\beta$ -CD in  $\text{DMSO}-d_6$ .

found for C = 44.62, H = 6.65, N = 5.28, and O = 43.53, which is related to a molecular formula of  $\text{C}_{486}\text{H}_{744}\text{N}_{50}\text{O}_{300} + 56\text{H}_2\text{O}$  corresponding to the PAMAM G1- $\beta$ -CD dendrimer with 56 molecules of water, this result is consistent with previous reports,<sup>61</sup> which indicates that in the cavities of the  $\beta$ -CD units water molecules are trapped.

#### Determination of the water solubility of the PAMAM G1- $\beta$ -CD dendrimer

The solubility of dendrimers is determined by the functional groups located in the periphery, by the generation of the dendrimer, by the repetitive units and even by the core. Their high water solubility guarantees their application as enhancers of the solubility of drugs with hydrophobic nature, as well as their application in the clinic area as drug carriers.<sup>62</sup> The conjugation of dendrimers with hydrophilic molecules, such as CDs, significantly increases their solubility in water and their stability, prolonging the blood circulation of these platforms.<sup>63</sup> The quantitative solubility in water of the PAMAM G1- $\beta$ -CD dendrimer was determined by the method reported by Jozwiakowski and Connors<sup>64</sup> and a result of  $620.5 \pm 0.0004 \text{ mg mL}^{-1}$  was obtained, which is considerably higher, compared to the solubility value of the native  $\beta$ -CD ( $18.5 \text{ mg mL}^{-1}$ ).<sup>65</sup> This result can be explained by the union of the PAMAM G1 dendrimer with the  $\beta$ -CD units, which generates a new dendritic structure with higher number of  $\beta$ -CD groups on the periphery, and is able to form hydrogen bonds with the aqueous medium. Another explanation for the enhanced solubility of PAMAM G1- $\beta$ -CD is that once the  $\beta$ -CD is linked to the PAMAM G1 dendrimer the intramolecular hydrogen bonds formed in the  $\beta$ -CD molecules alone, which are the cause of their low solubility,<sup>66</sup> are broken by mono-modification, giving rise to a better solubility in the new dendrimer. If the solubility of our dendrimer is

compared to the solubility values of some commercial  $\beta$ -CD derivatives, such as sulfobutylether- $\beta$ -CD ( $>500 \text{ mg mL}^{-1}$ ), O-methyl- $\beta$ -CD ( $>500 \text{ mg mL}^{-1}$ ) and 2-hydroxypropyl- $\beta$ -CD ( $600 \text{ mg mL}^{-1}$ ),<sup>65</sup> our dendrimer was found to have a higher solubility than all of them.

#### Cytotoxicity studies

To study the *in vitro* behavior of PAMAM G1- $\beta$ -CD dendrimer and corroborate that the design does not produce an undesirable toxic carrier, the cellular cytotoxicity of the PAMAM G1 and PAMAM G1- $\beta$ -CD dendrimers was evaluated in three different cancer cell lines; MCF-7, MDA-MB-231 and HeLa. Furthermore, and in accordance with our systematic evaluation, we also analyzed cytotoxic activity in a non-carcinogenic cell line, therefore LLC-PK1 was selected for this study. It has been reported that low-generation PAMAM dendrimers (G 0–2) do not present cellular toxicity.<sup>67–72</sup> To corroborate this behavior, we tested the cytotoxic activity of PAMAM G1 dendrimer in the four cell lines mentioned above. The results presented in Fig. S18† show that the PAMAM G1 dendrimer does not present any toxicity in the tested cell lines. Regarding the PAMAM G1- $\beta$ -CD dendrimer, in the carcinogenic and non-carcinogenic cell lines, six different concentrations from  $2 \mu\text{M}$  to  $100 \mu\text{M}$  were tested, taking a 100% of viability to untreated cells. In Fig. 2, we present the percentage of cell viability (%) versus the concentration of the tested PAMAM G1- $\beta$ -CD dendrimer; it is shown that in cancer cell lines and kidney cells, the PAMAM G1- $\beta$ -CD dendrimer does not show measurable cytotoxicity even at the highest evaluated concentration  $100 \mu\text{M}$ . This can be due to the fact that the primary amino groups on the periphery of the PAMAM G1 dendrimer are neutralized by the conjugation with the  $\beta$ -CD units and therefore no longer interact with the cell membrane, preventing it from compromising and eventually



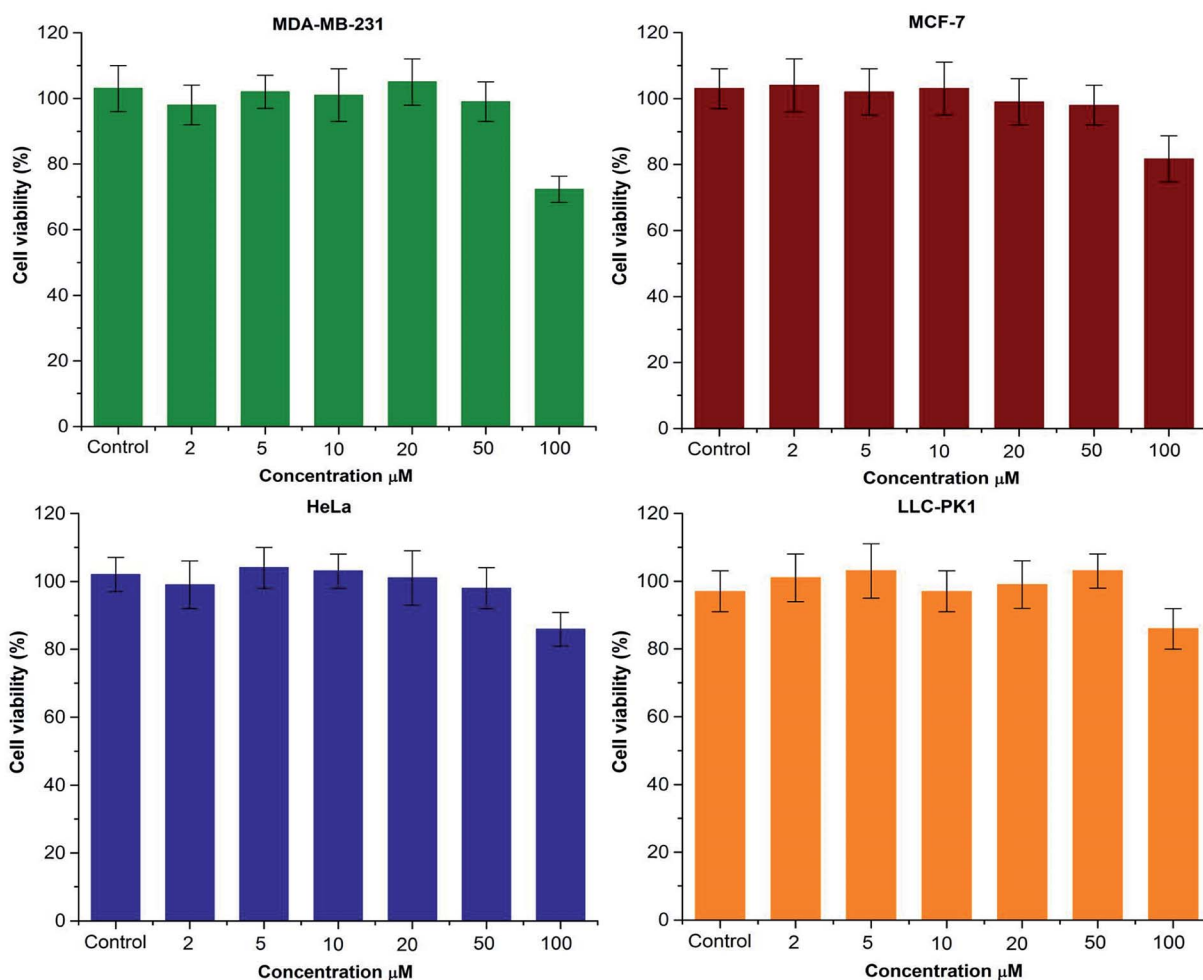


Fig. 2 Cell viability in different cell lines of PAMAM G1- $\beta$ -CD dendrimer. The treated cells were cultured in six wells by concentration, and the experiments were performed three times independently. Data are means  $\pm$  S.D.

causing cellular toxicity. If we compare Fig. 2 and S18,<sup>†</sup> we can assert that the complete modification of the periphery of the PAMAM G1 dendrimer with  $\beta$ -CD molecules produces a new non-toxic dendrimer.

## Conclusions

In this work, the complete modification of the periphery of the PAMAM G1 dendrimer with monofunctionalized  $\beta$ -CD molecules was successfully carried out. These macromolecules were coupled by a CuAAC reaction, giving the desired product with yields higher than 80% and a simple purification. The PAMAM G1- $\beta$ -CD dendrimer has been characterized by  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR and elemental analysis by X-ray fluorescence as a complementary way to characterize the modified PAMAM dendrimers. The water solubility of the PAMAM G1- $\beta$ -CD dendrimer was determined and resulted to be  $620.5 \text{ mg mL}^{-1}$ , considerably higher than native  $\beta$ -CD and even than other commercial CDs. Finally, we demonstrated that our PAMAM G1- $\beta$ -CD dendrimer does not present any cytotoxicity in the analyzed cell lines. Thus, this novel PAMAM G1- $\beta$ -CD dendrimer is a promising candidate to

be used in clinical applications as a possible drug carrier, which has the possibility of carrying larger units of drug molecules per carrier unit, with the advantage of having the synergy of the individual properties of the PAMAM dendrimer and the  $\beta$ -CD units.

## Experimental section

### Materials

All starting materials were commercially available reagent grade and were used without any further purification.  $\beta$ -Cyclodextrin ( $\beta$ -CD), PAMAM dendrimer G1 (PAMAM G1), propargyl bromide solution (80% by weight in toluene), *p*-toluenesulfonyl chloride (Cl-Ts), *p*-toluenesulfonic acid (OH-Ts), methyl 2-(4-hydroxyphenyl)acetate, *N,N*-dimethylformamide anhydrous (DMF), anhydrous potassium carbonate ( $\text{K}_2\text{CO}_3$ ), dimethylsulfoxide (DMSO), ascorbic acid ( $\text{H}_2\text{Asc}$ ), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl), Bio-Gel P-10 medium from BIO-RAD, 1-hydroxybenzotriazole hydrochloride (HOBt·HCl), *N,N*-diisopropylethylamina (DIPEA), potassium iodide (KI), sodium azide ( $\text{NaN}_3$ ), sodium hydroxide (NaOH),



copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), methanol (MeOH), ethanol (EtOH) and acetone ( $\text{CH}_3\text{COCH}_3$ ) were purchased from Sigma-Aldrich.

### Synthetic procedures

**Synthesis of methyl 2-(4-propargyloxyphenyl)acetate (I).** According to the method previously reported,<sup>73</sup> methyl-4-hydroxyphenyl acetate (5 g, 30.1 mmol),  $\text{K}_2\text{CO}_3$  anh. (5.5 g, 39.71 mmol) and propargyl bromide solution (4 mL, 80% in toluene, 30.1 mmol) were refluxed for 24 h; then the reaction mixture was cooled, filtered and the filtrate was evaporated. The residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (50 mL) and the solution was washed with water ( $2 \times 50$  mL). The organic layers were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and the solvent was evaporated under reduced pressure affording methyl-4-propargyloxyphenyl acetate as a brown oil (6 g, 29.4 mmol). Yield: 97.7%.  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.23 (d,  $J = 8.88$  Hz, 2H, c), 6.96 (d,  $J = 8.71$ , 2H, d), 4.69 (s, 2H, e), 3.71 (s, 3H, a), 3.59 (s, 2H, b), 2.54 (t, 1H, f).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 172.23, 156.70, 130.32, 127.04, 115.2, 78.57, 75.54, 55.83, 52.02, 40.28. ESI ( $m/z$ ): 204.08 [ $\text{M}^+$ ].

**Synthesis of 2-(4-propargyloxyphenyl)acetic acid (II).** Using a previously reported procedure,<sup>73</sup> we obtained 1.75 g (9.2 mmol) of 2-(4-propargyloxyphenyl)acetic acid as a yellow solid. Yield: 94%.  $^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 12.25 (s, 1H, a), 7.19 (m, 2H, c), 6.93 (m, 2H, d), 4.77 (d,  $J = 2.4$  Hz, 2H, e), 3.54 (t,  $J = 2.4$  Hz, 1H, f), 3.50 (s, 2H, b).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 173.38, 156.42, 130.82, 128.21, 115.08, 79.82, 78.58, 55.81, 40.21. ESI ( $m/z$ ): 191.07 [ $\text{M} + \text{H}^+$ ].

**Synthesis of alkynyl-PAMAM G1.** In a round flask, under anhydrous conditions, compound C (670.43 mg, 3.523 mmol), EDC·HCl (945.46 mg, 4.932 mmol), HOBt·HCl (539.44 mg, 3.523 mmol) and DIPEA (0.736 mL, 4.228 mmol) were dissolved in 12 mL of anhydrous DMF and the mixture was allowed to activate for 20 minutes. Then, a solution of PAMAM dendrimer G1 (450 mg/3 mL, 0.315 mmol) in DMF was added to the reaction mixture and stirred at 35 °C for 5 days. After this time, the DMF was evaporated under reduced pressure and the residue was recrystallized from MeOH, the final product was dried under vacuum. A brown oil was obtained (500 mg, 0.178 mmol). Yield: 57%.  $^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 8.48 (b, 8H, h), 8.24 (b, 12H, d), 7.18 (dd,  $J = 8.7$ , 3 Hz, 2H, j), 6.90 (dd,  $J = 8.7$ , 3 Hz, 2H, k), 4.76 (b, 16H, l), 4.11 (b, 16H, i), 3.55 (b, 8H, m), 3.17 (b, 32H, g), 3.11 (b, 24H, b), 3.05 (b, 8H, e), 2.98 (b, 8H, f), 2.89 (b, 24H, c), 2.33 (s, 4H, a).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 173.89, 158.84, 133.32, 117.81, 58.87, 51.49, 44.79, 41.71, 41.52, 39.61, 39.30, 28.48. MALDI-TOF ( $m/z$ ): 2810 [ $\text{M} + 3\text{H}^+$ ].

**Synthesis of mono-tosyl- $\beta$ -CD (m-Ts- $\beta$ -CD).** Following the procedure previously reported,<sup>74</sup> in a round flask, *p*-toluenesulfonic acid (1.918 g, 0.0101 mol) and *p*-toluenesulfonyl chloride (7.5 g, 0.039 mol) were dissolved in 50 mL of  $\text{CH}_2\text{Cl}_2$  and stirred at room temperature for 12 h. Then, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was recrystallized ( $3\times$ ) with cold hexane and the product ( $\text{Ts}_2\text{O}$ ) was filtered and allowed to dry under

vacuum. Afterwards, in a round flask,  $\beta$ -CD (5.75 g, 0.0051 mol) and  $\text{Ts}_2\text{O}$  (2.48 g, 0.0076 mol) were dissolved in 150 mL of  $\text{H}_2\text{O}$ . The reaction mixture was stirred for 2 h at room temperature; after this time, a 2.5 M aqueous NaOH solution was added. The reactions mixture was stirred for 10 minutes. Subsequently, the mixture was filtered, and the filtrates were adjusted to pH = 8 with a saturated solution of ammonium chloride to give a precipitate. The mixture was filtered, and the precipitate was recrystallized ( $3\times$ ) in acetone; a white solid was obtained: yield: 42% (2.7 g, 0.0021 mol).  $^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 7.75 (b, 2H) a, 7.44 (b, 2H) b, 5.83 (d,  $J = 6.4$  Hz, 1H) OH2', 5.78 (b, 6H) OH2, 5.71 (b, 7H) OH3, 4.84 (d,  $J = 3.9$  Hz, 6H) H1, 4.76 (d,  $J = 3.9$  Hz, 1H) H1', 4.50 (m, 6H) OH6, 4.35 (m, 2H) H6'ab, 4.19 (m, 1H) H5', 3.65 (m, 12H) H6ab, 3.60 (b, 7H) H3, 3.51 (m, 7H) H5, 3.30 (m, 7H) H2, 3.22 (m, 7H) H4, 2.42 (d, 3H) c.  $^{13}\text{C-NMR}$  (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 145.25, 133.09, 130.29, 128.03, 102.39, 101.74, 81.95, 81.21, 73.43, 73.16, 72.84, 72.48, 70.17, 69.36, 60.28, 21.62. MALDI-TOF ( $m/z$ ): 1311.591 [ $\text{M} + \text{Na}^+$ ].

**Synthesis of mono-azide- $\beta$ -CD (m- $\text{N}_3$ - $\beta$ -CD).** In a round flask m-Ts- $\beta$ -CD (2.120 g, 0.0016 mol),  $\text{NaN}_3$  (0.321 g, 0.005 mol), KI (0.137 g, 0.0008 mol) were dissolved in 8 mL of anhydrous DMF. The reaction mixture was stirred at 80 °C for 48 h. After this time, DMF was evaporated under reduced pressure and the residue was recrystallized in a mixture  $\text{H}_2\text{O} : \text{acetone}$  (1 : 1) and allowed to dry under vacuum. The yield of this reaction was 88% (1.7 g, 0.0014 mol) and a white solid was obtained.  $^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 5.74 (m, 7H) OH2, 5.67 (m, 6H) OH3, 5.62 (d,  $J = 2.4$  Hz, 1H) OH3', 4.88 (d,  $J = 3.5$  Hz, 1H) H1', 4.83 (m, 6H) H1, 4.48 (m, 6H) OH6, 3.77 (m, 2H) H6', 3.68 (m, 12H) H6, 3.60 (m, 7H) H3, 3.55 (m, 7H) H5, 3.39 (m, 7H) H4, 3.29 (m, 7H) H2.  $^{13}\text{C-NMR}$  (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 102.38, 102.04, 83.41, 81.99, 73.50, 73.30, 72.85, 72.67, 72.46, 70.63, 60.30, 51.53. MALDI-TOF ( $m/z$ ): 1182.764 [ $\text{M} + \text{Na}^+$ ].

**Synthesis of PAMAM G1- $\beta$ -CD dendrimer.** In a round flask, alkynyl-PAMAM (460 mg, 0.164 mmol), m- $\text{N}_3$ - $\beta$ -CD (1826.7 mg, 1573 mmol) and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (43.37 mg, 0.174 mmol) were dissolved, under nitrogen atmosphere, in 8 mL of DMSO. Then,  $\text{H}_2\text{Asc}$  (91.77 mg, 0.521 mmol) dissolved in 1 mL of DMSO was added and the reaction mixture was stirred at 80 °C for 48 h. After this time, the DMSO was removed under reduced pressure and the residue was precipitated with cold acetone, filtered and allowed to dry in vacuum. The product was purified by size exclusion chromatography (SEC), using Bio-Gel P-10, with water as solvent. A white solid (2.052 g, 0.169 mmol). Yield: 98%.  $^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 8.16 (m, 8H) triazole, 8.07 (m, 8H) 8, 8.02 (m, 12H) 4, 7.17 (m, 16H) 10, 6.97 (m, 16H) 11, 5.80 (m, 56H) OH2, 5.70 (m, 56H) OH3, 5.14 (m, 8H) H3', 4.88 (m, 16H) 12, 4.83 (m, 48H) H1, 4.75 (m, 8H) H1', 4.61 (m, 8H) H5', 4.55 (m, 48H) OH6, 4.48 (m, 16H) 9, 4.32 (m, 8H) H6'', 3.98 (m, 8H) H6', 3.69 (m, 96H) H6, 3.62 (m, 48H) H3, 3.57 (m, 48H) H5, 3.39 (m, 56H) H4, 3.29 (m, 56H) H2, 3.26 (m, 32H) 7, 3.10 (m, 24H) 2, 3.02 (m, 16H) 5, 2.88 (m, 16H) 6, 2.67 (m, 32H) 3, 2.34 (m, 4H) 1.  $^{13}\text{C-NMR}$  (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 130.57, 130.51, 115.04, 114.93, 102.38, 102.04, 83.42, 81.99, 73.50, 73.31, 72.88, 72.68, 72.49, 70.64, 60.34, 51.52. Elementary analysis: calculated for  $\text{C}_{486}\text{H}_{744}\text{N}_{50}\text{O}_{300}$  (C = 48.29, H = 6.20, N



= 5.79, O = 39.71). Found for  $C_{486}H_{744}N_{50}O_{300} + 56H_2O$  (C = 44.62, H = 6.65, N = 5.28, O = 43.53).

### Determination of solubility in water of PAMAM G1- $\beta$ -CD dendrimer

The determination of the solubility of the PAMAM G1- $\beta$ -CD dendrimer was carried out according to the method reported by Jozwiakowski and Connors<sup>64,75</sup> with some modifications. Excess amounts of the compound were placed in three amber vials of 5 mL with a screw cap, which were sealed with parafilm to avoid water evaporation. They were stirred in an oil bath at a constant temperature of  $25 \pm 0.01$  °C for 48 h. The supernatant was separated from the solid phase by filtration through a Milli-Q membrane (pore size of 0.45  $\mu$ m) by injection of the mixture into disposable plastic syringes of 3 mL at 25 °C. The supernatant of each sample was placed in three different vials. The samples were lyophilized for 48 h and the obtained solid was weighed on a scale with an uncertainty of  $\pm 0.0001$  g.

### Characterization

$^1H$ - and  $^{13}C$ -NMR measurements were carried out on a Bruker spectrometer (400 MHz), using DMSO- $d_6$  and  $CDCl_3$  as solvent. Chemical shifts are reported in ppm ( $\delta$ ) and the signals are described as singlet (s), doublet (d), triplet (t), quartet (q), broad (br), multiplet (m), and doublet of doublets (dd); coupling constants ( $J$ ) are reported in Hz. Mass measurements, ESI-MS and MALDI-TOF were performed on a JEOL JMS-AX505-HA instrument and on a Bruker Daltonics Flex Analysis instrument, respectively, matrices of 2,5-dihydroxybenzoic acid (DHB), dithranol (DIT) and 2-acetylphloroglucinol (THAP),  $AgNO_3$  and TFA as cationizing agents for the case of the PAMAM G1- $\beta$ -CD conjugate, were used for MALDI-TOF. The elemental analyses were recorded in an Ultra High Vacuum (UHV) of Physical Electronics, scanning XPS microprobe PHI 5000 VersaProbe II, with a MCD detector of 16 channel electron multipliers.

### Biological procedures

**Cell culture.** The human breast cancer cell lines MCF-7 and MDA-MB-231, human cervical adenocarcinoma cell line HeLa and the pig kidney epithelial cell line LLC-PK1 were selected for this study and purchased from the ATCC® (Manassas, VA USA). Cell lines were grown in D-MEM medium (Cellgro corning Manassas, VA USA) with 10 mM of non-essential amino acids, supplemented with 10% heat inactivated fetal bovine serum. These four adherent epithelial cell lines were maintained in proliferation under a humidified atmosphere containing 5%  $CO_2$  at  $37 \pm 1$  °C in a thermo scientific incubator model 3110 (Grand Island, NY, USA).

**Cytotoxicity studies.** To determine the cytotoxic activity of the PAMAM G1 and PAMAM G1- $\beta$ -CD dendrimers in the selected cell lines, the MTT assay was used.<sup>76</sup> Cell lines were briefly seeded in a 96-well plate at a density of  $6.5 \times 10^3$  cells per well and at a volume of 200  $\mu$ L of medium. After 24 h incubation, the cells formed a monolayer and 50  $\mu$ L of the medium with different concentrations of the dendrimer were added. The

treated cells were grown in six wells per concentration, and the experiments were performed three times independently. Cell viability was determined after 48 h exposure to the dendrimer by adding 20  $\mu$ L of 2.5 mg  $mL^{-1}$  3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Insoluble formazan, produced during an incubation period of 4 h at 37 °C, was measured. The supernatant was removed by solubilizing the formazan crystals formed by adding 250  $\mu$ L of DMSO. The absorbance of each well was measured at 550 nm using an Epoch microplate spectrophotometer (Biotek®, Winooski, VT, USA). Absorbance was directly related to percentage of cell viability (%), and control cells without treatment were considered 100% viability.

### Conflicts of interest

The authors declare no conflict of interest.

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### Notes and references

- 1 A. K. Sharma, A. Gothwal, P. Kesharwani, H. Alsaab, A. K. Iyer and U. Gupta, *Drug Discovery Today*, 2017, **22**, 314.
- 2 A. M. Caminade, *Chem. Commun.*, 2017, **53**, 9830.
- 3 B. K. Nanjwade, H. M. Bechra, G. K. Derkar, F. V. Manvi and V. K. Nanjwade, *Eur. J. Pharm. Sci.*, 2009, **38**, 185.
- 4 C. Diaz, C. Benitez, F. Vidal, L. F. Barraza, V. A. Jiménez, L. Guzman, J. Fuentealba, G. E. Yevenes and J. B. Alderete, *Nanomedicine*, 2018, **14**, 2227.
- 5 D. A. Tomalia, *Prog. Polym. Sci.*, 2005, **30**, 294.
- 6 P. Kesharwani, K. Jain and N. K. Jain, *Prog. Polym. Sci.*, 2014, **39**, 268.
- 7 P. K. Maiti, T. Çağın, S. T. Lin and W. A. Goddard, *Macromolecules*, 2005, **38**, 979.
- 8 D. Luong, P. Kesharwani, R. Deshmukh, M. C. I. Mohd Amin, U. Gupta, K. Greish and A. K. Iyer, *Acta Biomater.*, 2016, **43**, 14.
- 9 L. Albertazzi, M. Serresi, A. Albanese and F. Beltram, *Mol. Pharm.*, 2010, **7**, 680.
- 10 J. Yang, Q. Zhang, H. Chang and Y. Cheng, *Chem. Rev.*, 2015, **115**, 5274.
- 11 F. Gómez-Galván, L. Pérez-Álvarez, J. Matas, A. Álvarez-Bautista, J. Poejo, C. M. Duarte, L. Ruiz-Rubio, J. L. Vila-Vilela and L. M. León, *Carbohydr. Polym.*, 2016, **142**, 149.
- 12 D. Duchêne and A. Bochot, *Int. J. Pharm.*, 2016, **514**, 58.
- 13 G. Varan, C. Varan, N. Erdoğar, A. A. Hincal and E. Bilensoy, *Int. J. Pharm.*, 2017, **531**, 457.





- 14 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057.
- 15 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004.
- 16 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596.
- 17 H. T. Le, H. M. Jeon, C. W. Lim and T. W. Kim, *J. Pharm. Sci.*, 2014, **103**, 3183.
- 18 X. Wang, B. Huang, X. Liu and P. Zhan, *Drug Discovery Today*, 2016, **21**, 118.
- 19 N. M. Meghani, H. H. Amin and B.-J. Lee, *Drug Discovery Today*, 2017, **22**, 1604.
- 20 J. W. Lee, B.-K. Kim, H. J. Kim, S. C. Han, W. S. Shin and S.-H. Jin, *Macromolecules*, 2006, **39**, 2418.
- 21 X. H. Dai, W. H. Yang, W. L. Yan, J. M. Hu, Y. R. Dai, J. M. Pan and Y. S. Yan, *Colloids Surf., A*, 2017, **520**, 222.
- 22 G. Franc and A. Kakkar, *Chem. Commun.*, 2008, 5267.
- 23 Y. Kunsang, P. Goyal and M. Weck, *Org. Lett.*, 2007, **9**, 2051.
- 24 M. Malkoch, P. Wu, V. V. Fokin, E. Drockenmuller, C. J. Hawker, K. Schleicher and T. P. Russell, *Macromolecules*, 2005, **38**, 3663.
- 25 I. González-Méndez, A. Hameau, R. Laurent, C. Bijani, V. Bourdon, A. M. Caminade, E. Rivera and K. I. Moineau-Chane Ching, *Eur. J. Org. Chem.*, 2020, 1114.
- 26 H. Arima, F. Kihara, F. Hirayama and K. Uekama, *Bioconjugate Chem.*, 2001, **12**, 476.
- 27 A. F. A. Mohammed, T. Higashi, K. Motoyama, A. Ohyama, R. Onodera, K. A. Khaled, H. A. Sarhan, A. K. Hussein and H. Arima, *AAPS J.*, 2019, **21**, 1.
- 28 H. Arima and K. Motoyama, *Sensors*, 2009, **9**, 6346.
- 29 A. F. Abdelwahab, A. Ohyama, T. Higashi, K. Motoyama, K. A. Khaled, H. A. Sarhan, A. K. Hussein and H. Arima, *J. Drug Targeting*, 2014, **22**, 927.
- 30 H. Wang, N. Shao, S. Qiao and Y. Cheng, *J. Phys. Chem. B*, 2012, **116**, 11217.
- 31 S. Shah, A. Solanki, P. K. Sasmal and K. B. Lee, *J. Am. Chem. Soc.*, 2013, **135**, 15682.
- 32 H. Arima, Y. Chihara, M. Arizono, S. Yamashita, K. Wada, F. Hirayama and K. Uekama, *J. Controlled Release*, 2006, **116**, 64.
- 33 M. Labieniec-Watala and C. Watala, *J. Pharm. Sci.*, 2015, **104**, 2.
- 34 H. Arima, K. Motoyama and T. Higashi, *Pharmaceutics*, 2012, **4**, 130.
- 35 H. Arima, K. Motoyama and T. Higashi, *Adv. Drug Delivery Rev.*, 2013, **65**, 1204.
- 36 S. Menuel, S. Fontanay, I. Clarot, R. E. Duval, L. Diez and A. Marsura, *Bioconjugate Chem.*, 2008, **19**, 2357.
- 37 N. A. Stasko, C. B. Johnson, M. H. Schoenfish, T. A. Johnson and E. L. Holmuhamedov, *Biomacromolecules*, 2007, **8**, 3853.
- 38 T. Loftsson and M. E. Brewster, *J. Pharm. Sci.*, 1996, **85**, 1017.
- 39 Y. Zeng, P. Li, X. Liu, T. Yu, J. Chen, G. Yang and Y. Li, *Polym. Chem.*, 2014, **5**, 5978.
- 40 T. I. Al-Warhi, H. M. A. Al-Hazimi and A. El-Faham, *J. Saudi Chem. Soc.*, 2012, **16**, 97.
- 41 A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557.
- 42 N. Zhong, H. S. Byun and R. Bittman, *Tetrahedron Lett.*, 1998, **39**, 2919.
- 43 G. Tripodo, C. Wischke, A. T. Neffe and A. Lendlein, *Carbohydr. Res.*, 2013, **381**, 59.
- 44 Y. Toomari, H. Namazi and E. A. Akbar, *Carbohydr. Polym.*, 2015, **132**, 205.
- 45 K. S. Anseth and H.-A. Klok, *Biomacromolecules*, 2016, **17**, 1.
- 46 M. P. Lutolf and J. A. Hubbell, *Biomacromolecules*, 2003, **4**, 713.
- 47 J. Fang, S. H. Ye, J. Wang, T. Zhao, X. Mo and W. R. Wagner, *Biomacromolecules*, 2015, **16**, 1622.
- 48 R. T. Chen, S. Marchesan, R. A. Evans, K. E. Styan, G. K. Such, A. Postma, K. M. McLean, B. W. Muir and F. Caruso, *Biomacromolecules*, 2012, **13**, 889.
- 49 N. J. Agard, J. A. Prescher and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 15046.
- 50 G. Yi, J. Son, J. Yoo, C. Park and H. Koo, *Biomater. Res.*, 2018, **22**, 1.
- 51 S. Srinivasachari, K. M. Fichter and T. M. Reineke, *J. Am. Chem. Soc.*, 2008, **130**, 4618.
- 52 B. Parrish, R. B. Breitenkamp and T. Emrick, *J. Am. Chem. Soc.*, 2005, **127**, 7404.
- 53 C. Zhang, D. Pan, K. Luo, N. Li, C. Guo, X. Zheng and Z. Gu, *Polym. Chem.*, 2014, **5**, 5227.
- 54 A. Ghilardi, D. Pezzoli, M. C. Bellucci, C. Malloggi, A. Negri, A. Sganappa, G. Tedeschi, G. Candiani and A. Volonterio, *Bioconjugate Chem.*, 2013, **24**, 1928.
- 55 J. H. Lee, Y. B. Lim, J. S. Choi, M. U. Choi, C. H. Yang and J. S. Park, *Bull. Korean Chem. Soc.*, 2003, **24**, 1637.
- 56 E. Arnáiz, L. I. Doucedo, S. García-Gallego, K. Urbiola, R. Gómez, C. Tros De Ilarduya and F. J. De La Mata, *Mol. Pharm.*, 2012, **9**, 433.
- 57 J. E. Hein and V. V. Fokin, *Chem. Soc. Rev.*, 2010, **39**, 1302.
- 58 L. J. López-Méndez, I. González-Méndez, R. Aguayo-Ortiz, L. Dominguez, S. L. Alcaraz-Estrada, Y. Rojas-Aguirre and P. Guadarrama, *Carbohydr. Polym.*, 2018, **184**, 20.
- 59 J. Peterson, V. Allikmaa, J. Subbi, T. Pehk and M. Lopp, *Eur. Polym. J.*, 2003, **39**, 33.
- 60 S. M. Weidner, U. Just, W. Wittke, F. Rittig, F. Gruber and J. F. Friedrich, *Int. J. Mass Spectrom.*, 2004, **238**, 235.
- 61 D. Harries, D. C. Rau and V. A. Parsegian, *J. Am. Chem. Soc.*, 2005, **127**, 2184.
- 62 Y. Cheng, Z. Xu, M. Ma and T. Xu, *J. Pharm. Sci.*, 2008, **97**, 123.
- 63 S. Mignani, S. El Kazzouli, M. Bousmina and J. P. Majoral, *Prog. Polym. Sci.*, 2013, **38**, 993.
- 64 M. J. Jozwiakowski and K. A. Connors, *Carbohydr. Res.*, 1985, **143**, 51.
- 65 M. E. Brewster and T. Loftsson, *Adv. Drug Delivery Rev.*, 2007, **59**, 645.
- 66 T. Loftsson and D. Duchêne, *Int. J. Pharm.*, 2007, **329**, 1.
- 67 N. Malik, R. Wiwattanapatapee, R. Klopsch, K. Lorenz, H. Frey, J. Weener, E. Meijer, W. Paulus and R. Duncan, *J. Controlled Release*, 2000, **65**, 133.
- 68 R. Dong, L. Zhou, J. Wu, C. Tu, Y. Su, B. Zhu, H. Gu, D. Yan and X. Zhu, *Chem. Commun.*, 2011, **47**, 5473.



- 69 M. El-Sayed, C. A. Rhodes, M. Ginski and H. Ghandehari, *Int. J. Pharm.*, 2003, **265**, 151.
- 70 F. Tajarobi, M. El-Sayed, B. D. Rege, J. E. Polli and H. Ghandehari, *Int. J. Pharm.*, 2001, **215**, 263.
- 71 J. Auclair, E. Morel, K. J. Wilkinson and F. Gagné, *EC Pharmacol. Toxicol.* **3.5**, 2017, 138–145.
- 72 R. Duncan and L. Izzo, *Adv. Drug Delivery Rev.*, 2005, **57**, 2215.
- 73 S. Rahmani and A. S. zadeh Mahani, *Macromol. Res.*, 2015, **23**, 1018.
- 74 N. Zhong, H. S. Byun and R. Bittman, *Tetrahedron Lett.*, 1998, **39**, 2919.
- 75 E. Sabadini, T. Cosgrove and F. D. C. Egídio, *Carbohydr. Res.*, 2016, **341**, 270.
- 76 I. González-Méndez, J. D. Solano, P. Porcu, A. Ruiu, Y. Rojas-Aguirre and E. Rivera, *J. Mol. Struct.*, 2019, **1177**, 143.

