Recent Advances of Dendrimers in Delivery of Genes and Drugs

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Abstract: Dendrimers as a new kind of polymer have been studied for medical applications mainly in two aspects: drug delivery and gene delivery. The unique characteristics, such as uniformity, monodispersity and the ability to functionalize their terminal groups with various targeting agents distinguish them as versatile carriers. In the paper the recent advances of dendrimer in gene transfer vehicles and drug delivery are separately reviewed. These advances illustrate the direction of the future development of dendrimers.

Key Word: Dendrimer, delivery of dene, delivery of drug, DNA/RNA, PAMAM, PEI, PPI.

1. INTRODUCTION

In 1985, Tomalia *et al*. [1] synthesized for the first time a new kind of polymer which they referred to as "starburst polymer". Because of its dendritic architecture, people use the term "dendrimer" to describe this new class of polymers thereafter. Interest in dendrimers has grown steadily over the past two decades to use these molecules in numerous industrial and biomedical applications [2]. In 2003, the first dendrimer drug developed by StarPharma® (Melbourne, Australia) for use against HIV, received regulatory clearance for phase (I) clinical trials from the US FDA. The drug is a topical gel containing an anionic polyamidoamine dendrimer which is postulated to interfere with the entry and fusion process of the HIV virus [3]. However, in biomedical science, dendrimers were studied mostly as delivery agents in both gene and drug delivery systems rather than as therapeutic agents.

In 1993, Szoka *et al*. [4] showed that dendrimers could be used as vehicles to introduce gene into cell. Since then, many researches focus on it. In 1996, Szoka and co-workers showed that fractured dendrimers led to enhanced DNA transfection [5], thus led to a commercially available transfection agent Superfect™, a fractured PAMAM (polyamidoamine) dendrimer. In gene delivery applications, existing gene transfer systems, such as viral vectors (retroviral and adenoviral vectors etc.) or nonviral vectors (plasmids, liposomes etc.), pose problems mainly because of mutagenesis, immunogenicity, inflammation, and low targeting efficiency [6].

Dendrimers have primary amine end groups, which could participate in DNA binding processes. They could be linked to various biocompatible molecules for different applications [7, 8]. In addition, while most of liposomes are considered energetically metastable and will eventually rearrange to form planar bilayers, dendrimers are considered more stable [9, 10]. Dendrimers with a high density of charged primary amino groups restricted to the surface are highly soluble which makes them stable in aqueous solution. On the other hand, studies have shown that dendrimers are nonimmunogenic and can mediate the enhanced delivery of diverse nucleic acids, including DNAs and RNAs [11, 12].

In drug delivery, because of the unique characteristics such as uniformity and monodispersity, dendrimers possess the potential to be used as carriers for targeted drug delivery due to their terminal groups could be functionalized with various targeting therapeutic and imaging agents in specific and controllable manner [2, 13, 14]. Compared to another widely used material in biomedical applications, liposome, which has been under development as delivery vehicles in both gene and drug delivery systems since early 1990s, dendrimers are well defined in size and capable of loading drugs on their surface or encapsulating them within. They have higher delivery efficiencies and lower drug leakage in drug delivery applications [15].

All these advantages of dendrimer in drug delivery and gene delivery lead researchers to expect that it could replace liposomes and other carrier in some practical uses. It indicates that dendrimers begin to play a more and more important role in practical applications. Although dendrimers have many advantages in gene and drug delivery, they still cannot entirely replace other delivery systems. The suitability of any delivery system should always be matched with the clinical situation, the specific disease and the chosen therapeutic strategy [16].

2. RECENT ADVANCES IN GENE DELIVERY

Different kinds of nucleic acids can be used for gene delivery experiments, including DNA/RNA, and oligonucleotides. All these nucleic acids face the same barriers when transfected. In the last 10 years, there has been an explosion of interest in using dendrimers as vectors [17]. Researches showed that dendrimers, especially PAMAM dendrimers, allowed efficient transferring in many different cell types and cell lines [4, 18, 19]. PAMAM were studied more intensively than any other dendrimers, like PEI (poly(ethyleneimine)), PPI (poly(propylene imine)) and their derivatives [20]. Several different formulations of the dendrimer–DNA

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complex were investigated, such as PAMAM/DNA [4], PEG (polyethylene glycol)-PAMAM/DNA [21], PAMAM-PEG-PAMAM/DNA [22], PPI/DNA [23], PEI/DNA [24].

In mechanism terms, it appears that dendrimer is in fact quite similar to other gene delivery agents in general but not in details. It contains two processes: 1. the formation of complexes with DNA/RNA; 2. the facilitated entrance of the complexes into the cells [25].

2.1. Enhancement of DNA Complex Formation

The original polyamidoamine in high generation (>G5) has a sphere shape and these dendrimers represent the first family to be made commercially available and it was widely studied in its spontaneous ability to form complexes with DNA/RNAs. Based on the research of the mechanism of the formation process known so far [26-28], the cationic charged primary amino group on the surface can easily attach to the DNA chain, and then attract the DNA chain to wrap around it by electrostatic force. The shape and the density of charged groups on the surface may be the most important factors in the formation process. Changing the shape of the PAMAM dendrimer could lower the transition energy and make it easier to form complexes with the DNA/RNA chain. Recently, Kim group [29] found another direct evidence of the interaction between PAMAM and DNA probed by Hoechst 33258 (Fig. **1**).

Their data obtained from Linear Dichroism (LD) showed that a large part of linear DNA wrapped the surface of G-6 dendrimer as the previous research suggested [30-32]. The interaction between the DNA and G-6 dendrimer was correlated to the shape of the dendrimer.

2.2. Promotion of Cell Entrance

In the uptake process, dendrimer facilitates the entrance of the complexes by an initial electrostatic attraction between the cationic complex and the negatively charged cell surface groups. DNA-dendrimer complexes transit the cell membrane or the endosomal membrane by endocytosis, and finally transport into the nucleus by an endosomal escape mechanisms [25]. Dendrimer could protect the DNA apart from cellular nucleases and degradation. Recent research suggested that the binding and uptake of dendrimer depended on cholesterol [33]. Another research based on various measurement methods showed that there are no obvious correlations with gene delivery activity and the physical properties. However, according to the previous research on PAMAM dendrimers [34], dendrimer/DNA complexes with mean diameter (nm)/zeta potential (mV) ratio between 4 to 8 are more efficiency.

2.3. Modification of Head-Tail Configuration

In the recent research on gene delivery by Atsushi Harada *et al*. [35], a head-tail type of polycation block copolymer was synthesized based on the structure of PAMAM (Fig. **2**). Dendrimers with a long PEG tail or linked by a long PEG chain (like a barbell) have been synthesized and presented low cytotoxicity and high solubility [22, 36, 37]. The previous research on this kind of dendrimer found that the large head with a strong hydrophilic linear tail tended to gather on the air/water interface more efficiently [38]. The newly synthesized PAMAM dendron-PLL (poly(L-lysine)) polyplexes shows high gene delivery efficiency in HeLa cells.

They believed that the structure of the tail block complexed with pDNA and the head block would have a buffering effect that made the PAMAM dendron-PLL polyplexes get a 10^2 fold higher gene delivery efficiency in HeLa cells as that of PLL polyplexes.

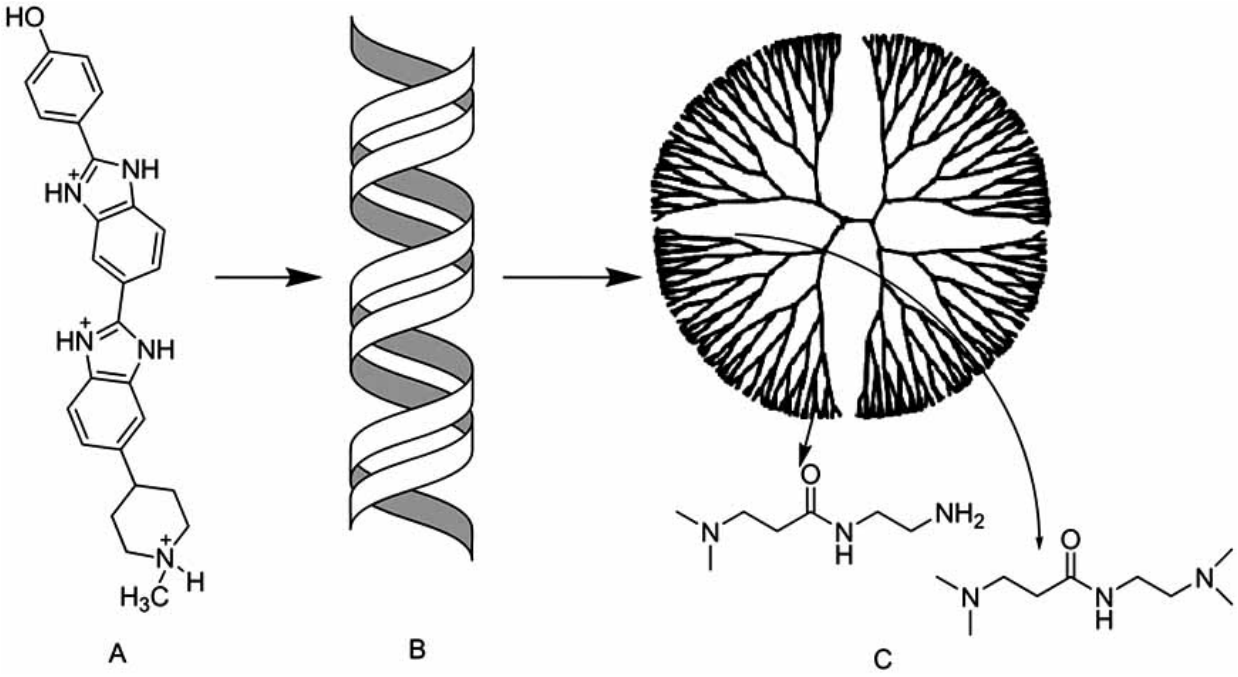


Fig. (1). Molecular structure of Hoechst 33258 (A) which could bound to the minor groove of DNA (B) and indicate the interaction between DNA and PAMAM dendrimer (C).

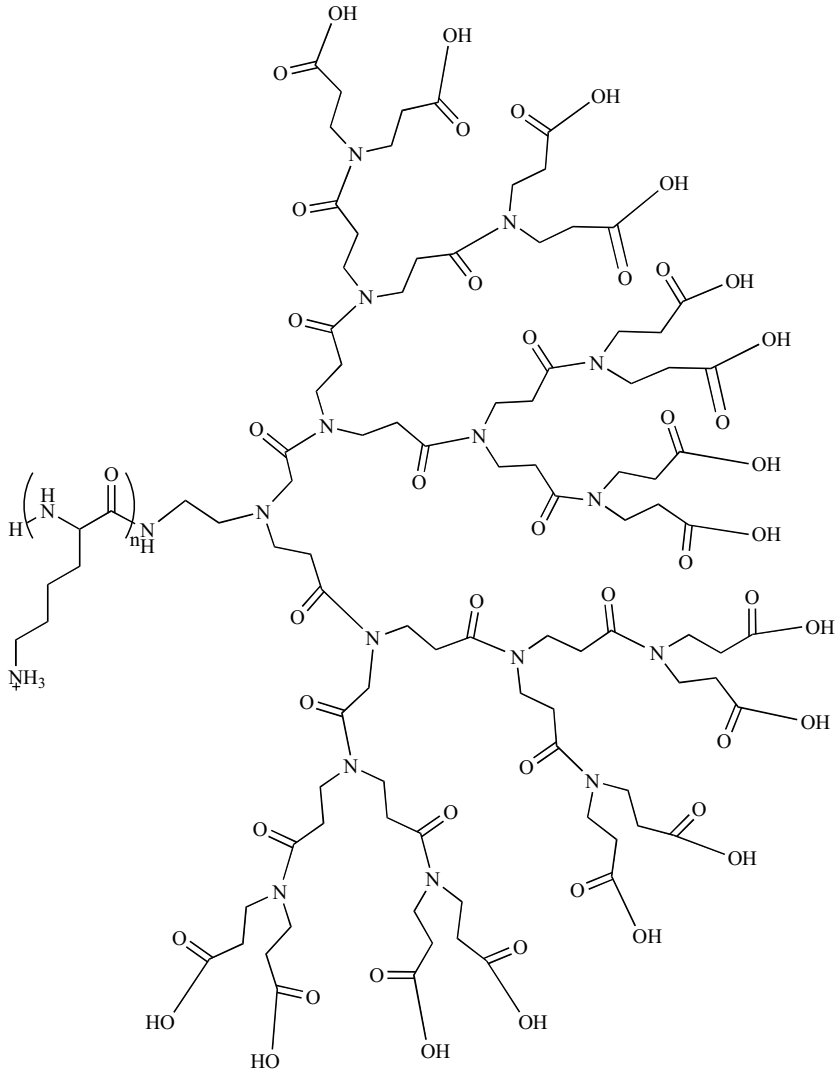


Fig. (2). Structure of newly synthesized head-tail polycation block copolymer by Harada *et al*. as nonviral gene vector.

2.4. Introduction of PCI (Photochemical Internalization)

To enhance gene delivery efficiency and specificity, external stimuli may be a promising approach for the site-directed gene delivery *in vivo*. A new approach called "photochemical internalization" (PCI) was introduced by Høgset and Berg *et al*. [39] in 1999 to overcome both the limited gene delivery efficiency and the lack of specificity of non-viral gene vectors. This new approach depends on a light activation of photosensitizers located in the membranes of endosomes and lysosomes to destroy the membranes, whereas the contents of the organelles remain intact, and in this way the endocytosed macromolecules are delivered into the cytosol. Nishiyama *et al*. [40] used a PEG-PLL copolymer to form polymeric micelles and a dendrimer DPc (dendrimer phthalocyanine) (Fig. **3**) as a photosensitizer in the PCI-mediated gene delivery *in vitro* in HeLa and HUH-7 cells. A series of samples with different N/P ratios and DPc concentrations were used in this study. The results showed that polymeric micelles played an important role in many aspects, such as prolonged blood circulation and enhanced stability. The highest gene expression was achieved at an N/P ratio of 1.2 and DPc concentration at 3.2×10^{-7} M. The photochemical enhancement of luciferase gene expression could reach 56 - 212 folds high in the presence of light irradiation (fluence: 5.4 J/cm^2). Compare to the DPc without copolymer micelles, the photochemical enhancement and the cell viability increased remarkably.

2.5. Surface Modification

Tack *et al*. [41] used various modified poly (propylene imine) (PPI) dendrimer to delivery a 33-mer oligonucleotide (DNAzyme) *in vitro* and *in vivo*. PPI-dendrimer has been modified firstly at the exterior primary amines with acetyl groups or PEG-like groups, and then at the interior tertiary amines with methyl halogen to produce multiple quaternized cationic sites in the core of the dendrimer (Fig. **4**). Polyacrylamide gel electrophoresis (PAGE) was performed to investigate the binding properties of the PPI-DNA complex. The results demonstrated a strong but reversible binding especially in the quarternized and higher generation dendrimer

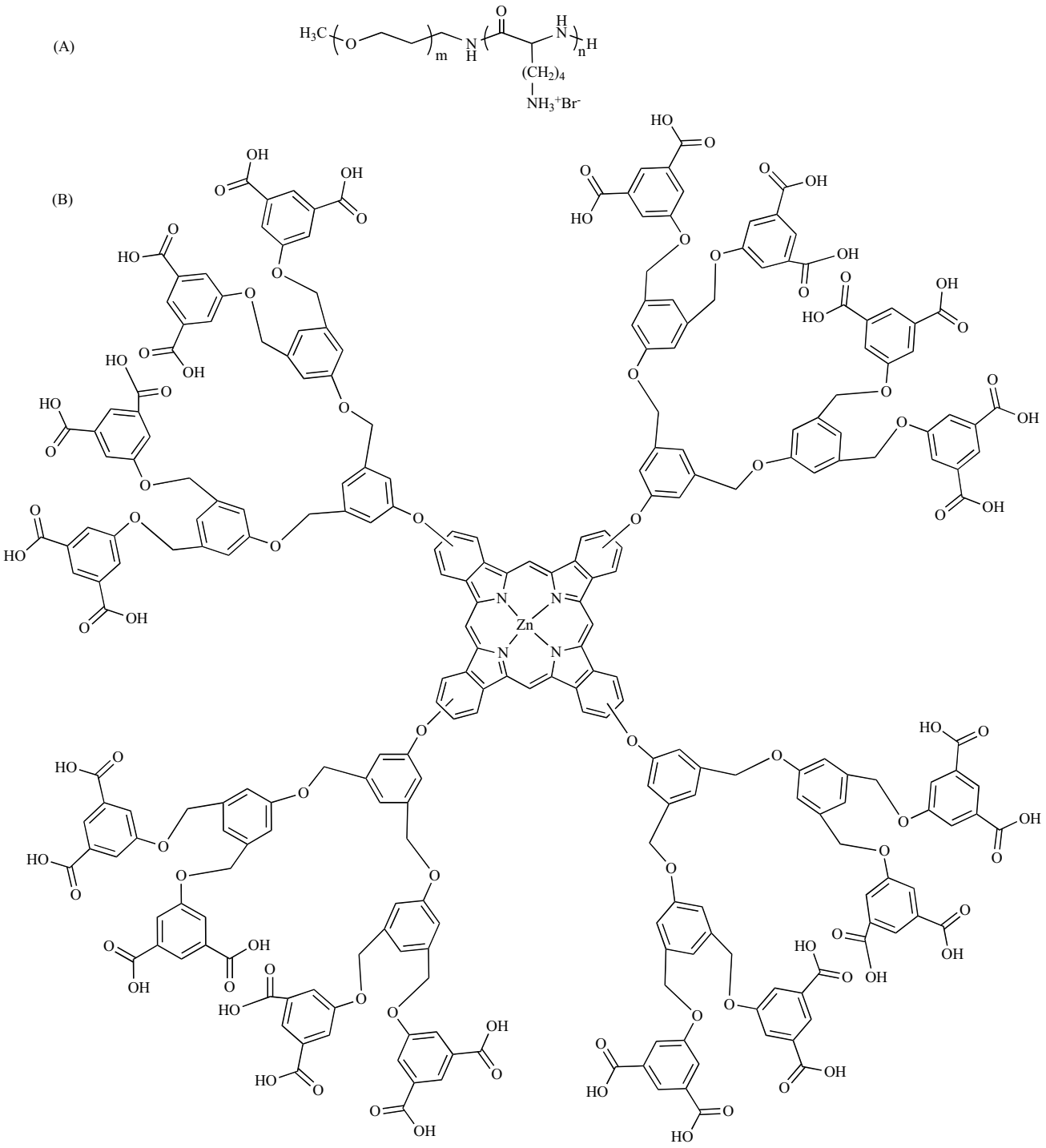


Fig. (3). Chemical structures of PEG–PLL block copolymer (A) and DPc (B).

species. Binding was observed at a concentration of about 4 μM DNA and a dendrimer-DNA charge ratio of around 2:1–1:1 in a previous research. The fluorescence correlation spectroscopy (FCS) of the complexation between oligonucleotides and PPI indicated that at low N/P ratio, oligonucleotides and dendrimer tended to form "multimolecular complexes" while "mono-molecular complexes" were formed at high N/P ratio [42]. All the tested PPI-dendrimer displayed a low cellular toxicity and high gene delivery efficiencies (close to 80%). The formation of the quaternary amine group in PPI decreased the cytotoxicity, just as the previous research showed in 293T cells [43]. *In vivo* experiment of co-

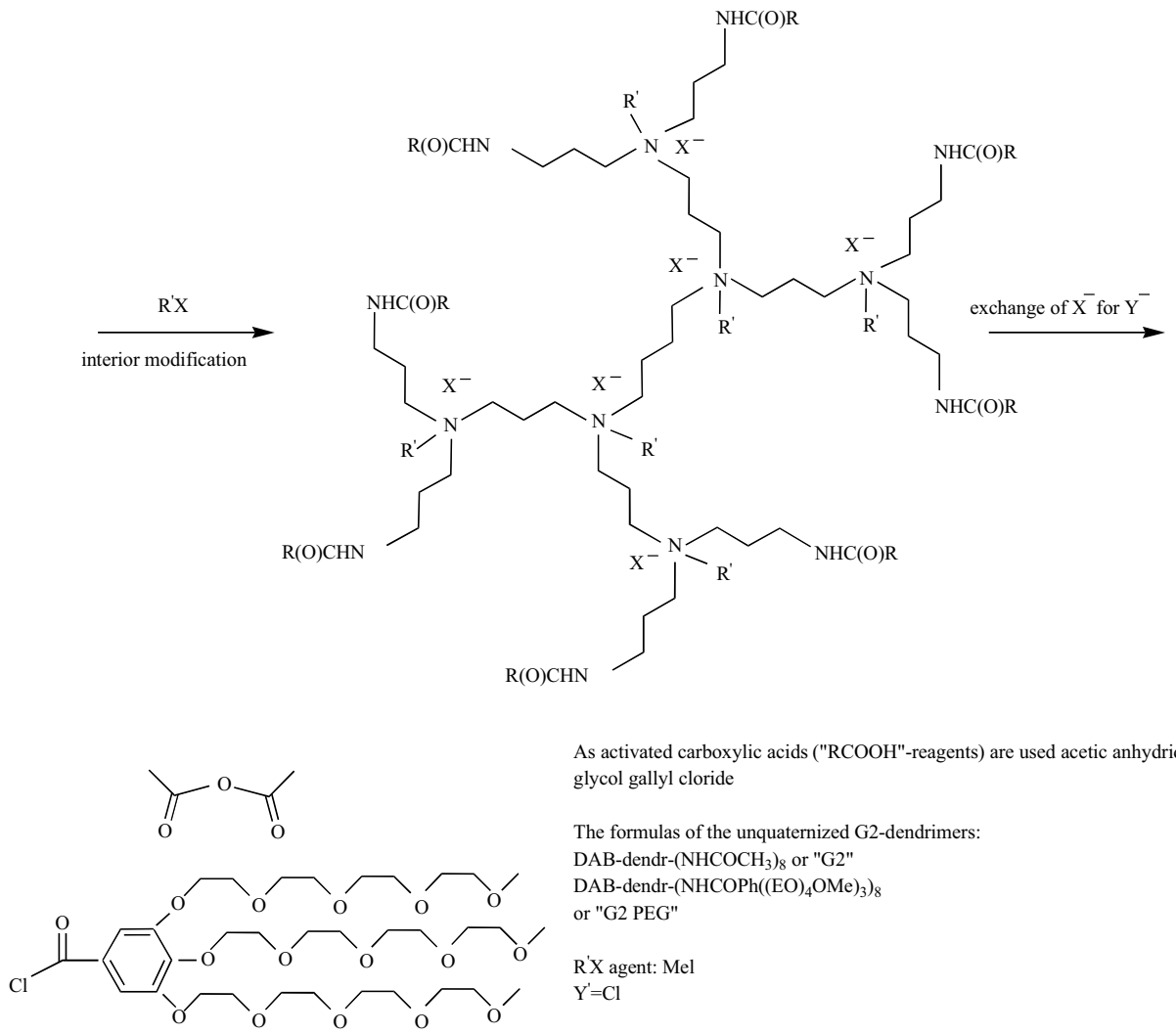


Fig. (4). A. The applied synthesis of cationically modified PPI-dendrimer as illustrated using the second generation PPI-dendrimer. In the first step the exterior primary amines are amidated using activated carboxylic acid derivatives ("RCOOH"), while in the second step the interior tertiary amines are alkylated to produce positively charged quaternary ammonium sites. In the third step, the anion is exchanged. B. In the box the various R-, R'-, X- and Y-groups are shown that have been used in this study [41].

localization studies displayed high nuclear uptake when the G4-PEG (MeI)–ssDNA complex was administered i.v. of Nude mice .

2.6. Dendrimer with Intrinsical Fluorescence

A new kind of intrinsically fluorescent dendrimer was recently synthesized by Al-Jamal *et al.* [44] (Fig. **5**). The intrinsical fluorescence could be detected at low concentrations in the investigation of dendrimers uptake by cells (Fig. **6**) instead of the fluorescent dye, which has been used previously. This kind of cationic dendrimer is based on lysine and lack fluorophores. The nomenclature used to describe these kinds of compounds results in, for example the 6th generation dendrimer being notated as Gly–$Lys_{63}(NH_2)_{64}$; Gly denotes that the compound has a glycine in the core coupled to 63 lysine branching units (Lys_{63}) and that the surface has 64 free amino groups $(NH_2)_{64}$. The lysine-based dendrimer has been studied in forming complexes with DNA and heparin [45, 46]. This kind of dendrimers with high gene delivery efficiency and cell viability could protect the DNA from nuclease degradation.

In vitro experiment on Caco-2 cells showed a clear view of the uptake mechanism of the entrance of these kinds of dendrimers (Fig. **6**).

In Fig. (**6A**), cells were incubated without dendrimer. In Fig. (**6B** and **C**), cells were incubated with dendrimers for 15 min and 30 min, and the dendrimer was attached to the membrane (red arrow) by electrostatic attraction. In Fig. (**6 D**), cells were incubated with dendrimers for 1h, and it showed that dendrimers were distributed all around the cells. The uptake of dendrimer–DNA complexes by living cells is being studied using the same technique.

2.7. Other Advances

Some other recent progresses in gene delivery were mainly concentrated on the amino acid grafted surfaces

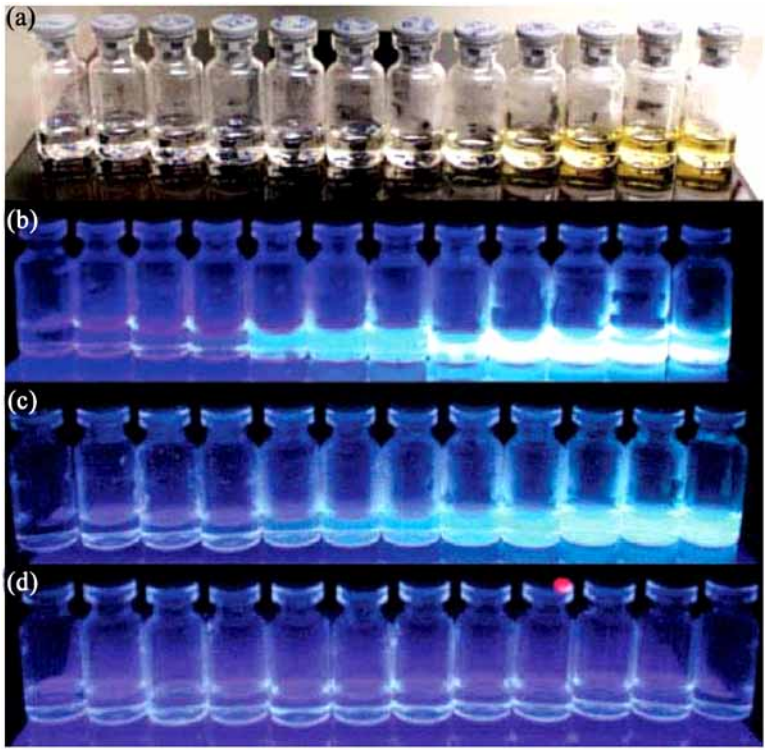


Fig. (5). Photographs of a range of concentrations (0.01–3mM) of (a) Gly–Lys_{63} $(NH_2)_{64}$ (0.08–24.5 mg/mL) exposed to visible light; (b) Gly–Lys_{63} $(NH_2)_{64}$ (0.08–24.5 mg/ml); (c) Gly–Lys_{31} $(NH_2)_{32}$ (0.04–12.14mg/mL); and (d) polylysine hydrobromide (MW 9600 Da) (0.096–28.8 mg/mL) exposed to a UV transilluminator. Fluorescence increased with the dendrimer concentration and generation number (b, c). Linear polylysine does not show intrinsic fluorescence behavior (d).

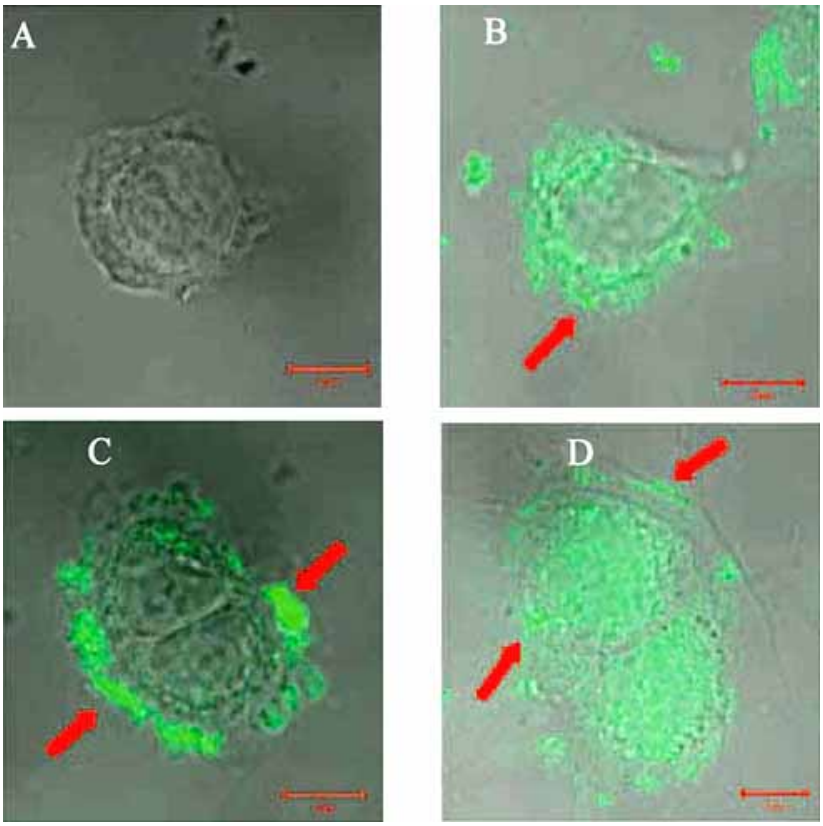


Fig. (6). A time dependent uptake study of the dendrimer (green fluorescent) in fixed Caco-2 cells.

(arginine, spermine, etc.) which could be helpful on the formation of more stable DNA-dendrimer complexes and on facilitating the uptake process [47-49]. The complexes of metal ion (gadolinium, platinum, ruthenium, etc.) with dendrimers, which could be used in the MRI (magnetic resonance imaging) as contrast agents, were also reported to form complexes with DNA. [50-52].

3. NEW PROGRESS IN DRUG DELIVERY

Dendrimer was used in drug delivery mainly to improve the solubility, biocompatibility and pharmacokinetic properties of drugs. The advantage of dendrimer lies in the endless diversity in topology and chemistry modifications. The original dendrimers (PAMAM, PPI, PEI and their derivatives) in high generations rarely used directly in drug delivery systems due to their cytotoxicities [53, 54]. Dendrimers commonly used in drug delivery are modified mainly in three ways: 1. PEGylation, 2. FA (folic acid) receptor targeting groups, 3.Stimuli-sensitive function groups.

PEGylated (polyethylene glycol) dendrimer is a big family since various PEG-grafted dendrimers have been synthesized for many different purposes. PEG is often attached to dendrimers to impart aqueous solubility [55-58], biocompatibility [22, 59, 60], and to increase drug loading [56]. PEG has a noticeable ability to enhance the solubility of some drug which lacks hydrophilic groups like adriamycin [59], artemether [56], diclofenac [57], 5-fluorouracil [61], indomethacin [62], and methotrexate [59]. Further more, some chemical modifications on the PEGylate dendrimers could achieve controlled release of drugs [57, 63].

PEG as a nonimmunogenic and biocompatible material was often introduced to reduce the toxicity of original dendrimers. In an *in vivo* test, PEGylated dendrimers showed low toxicity even in high doses up to 2.56 g/kg i.p. and 1.28 g/kg i.v. [60] in Male C3H mice. Linear copolymers in an ABA or AB pattern seem to be useful to enhance the drug loading. PEGylated lysine [56] and PEG based glutamic acid copolymer [64] in an AB pattern enhanced the drug loading remarkably in artemether (antimalarial drug) and epirubicin (antitumor drug). A recent study on an ABA pattern copolymer [65], PAMAM-PPO(poly(propylene oxide))-PAMAM, showed high drug loading capacity in triclosan (bactericide), and the total loading capacity increased as the generation of the PAMAM dendrimer increased, and the highest encapsulation (w/w) reached by G4 PAMAM copolymer with 86%.

Another big family of grafted dendrimer used in drug delivery is the FA-dendrimer family. Folic acid was used to target dendrimers to tumor cells which are the high-affinity folate receptor [66]. FA-dendrimer complexes were widely studied *in vitro* [67-69] and *in vivo* [70]. Various anticancer drugs were studied to form FA-dendrimer complexes for targeting delivery into different tumor cell lines. Methotrexate (MTX) (a toxic antimetabolite drug, which acts as a folic acid antagonist to interfere with cellular reproduction in the treatment of certain cancers) was attached to FA-dendrimers in many different works for specific targeting. FA–dendrimer-methotrexate complexes were firstly synthesized in 1999 [71], and the complex could target the MTX to the specific FA receptor on the tumor cells [72-75]. The newest research in both MTX-sensitive and MTX-resistant cell lines showed that FA targeted dendrimer with MTX might achieve highly therapeutic effects even in MTX-resistant cells [76]. The FA-dendrimer complex was also used to detect tumor by adding a near-infrared fluorochrome [77].

In the biological systems, numerous pH and temperature gradients exist in both normal and pathophysiological tissues. Stimuli-sensitive dendrimers include dendrimers that are responsive to stimuli such as changes in pH [78-80] or temperature [81, 82]. A noticeable research by Gillies *et al.* showed that the synthesized a group of dendrimer could form stable micelles in pH 7.4 (blood and normal tissues) and dissociated at pH 5.0 (tumors), and the drugs encapsulated in the micelles would release smoothly [78]. In addition, antibodies could also cause the initiative release. A family of self-immolative endrimers were designed and synthesized by Shabat *et al.* [83, 84]. These dendrimers contained a retro-aldol retro-Michael focal trigger, which could be cleaved by catalytic antibody 38C2.

3.1. Hydrophilic Interior Dendrimer

Dhanikula and Hildgen [85] synthesized a series of dendrimers with a hydrophilic interior, which demonstrated a good ability to encapsulate the guest molecule inside, with a loading of 15.80% and 6.47% w/w for rhodamine and *β*-carotene, respectively. A similar structure of such a hydrophilic interior was synthesized and measured before without drug load-release experiments [86]. The core was synthesized from biocompatible moieties, butanetetracarboxylic acid and aspartic acid, and the dendrons from PEO (poly (ethylene oxide)), dihydroxybenzoic acid or gallic acid, and PEG monomethacrylate. PEO was incorporated in the interior of the dendrimers to increase the size of the cavity as well as to provide a hydrophilic interior region, (Fig. **7**).

This kind of dendrimer demonstrated the ability to encapsulate both hydrophilic and hydrophobic model compounds in the encapsulation and release studies. It showed that 90% of drug was smoothly released in 170 h (Fig. **8**).

The study shows that PEO interior could achieve a stable controlled release not only in the hydrophobic drugs but also in the hydrophilic drugs.

3.2. Multifunctional Dendrimer

István *et al*. designed and synthesized PAMAM-dendrimer-based multifunctional cancer therapeutic conjugates which could deliver drugs to specific receptors on cancer cells [87]. Paclitaxel (Taxol®, a chemotherapeutic drug) was attached to a G5-PAMAM in conjugation with FITC (fluorescein isothiocyanate, an imaging agent) and folic acid which targeted overexpressed folate receptors on specific cancer cells. The primary amino groups on the surface of the G5-PAMAM were neutralized through partial acetylation, providing enhanced solubility of the dendrimer and preventing nonspecific targeting interactions (*in vitro* and *in vivo*) during delivery. Attachment of glycidol was a necessary precursory step to the attachment of Taxol® through an ester linkage, (Fig. **9**).

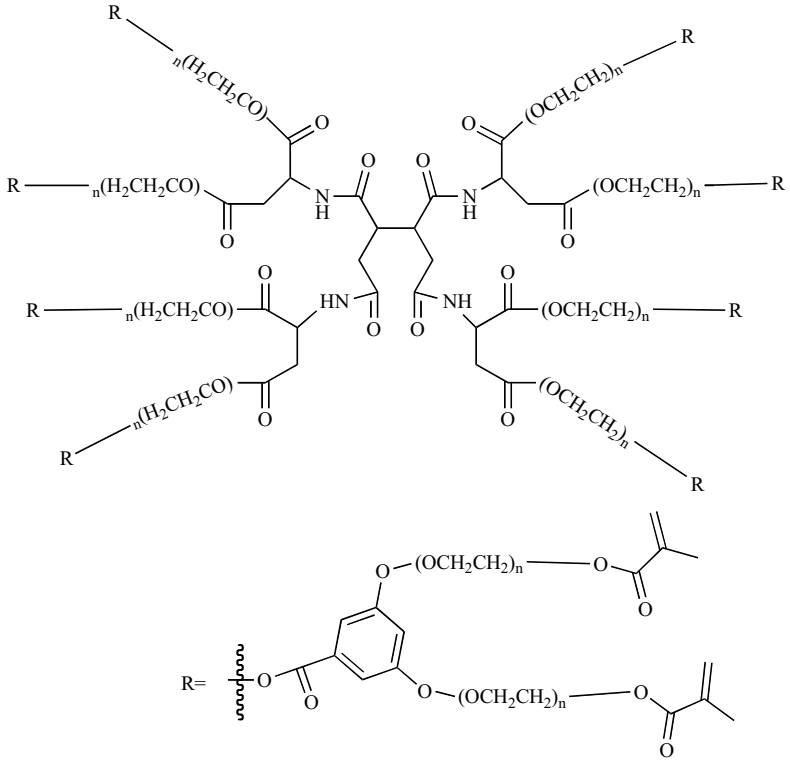


Fig. (7). Hydrophilic interior dendrimer generation 2 with a butanetetracarboxylic acid core and dendrons from PEO and gallic acid.

The *in vitro* studies showed that only KB cells with up-regulated folate receptor and treated with the trifunctional dendrimer conjugate displayed green fluorescence, demonstrating the uptake of the dendrimer conjugate. The folate receptor down-regulated KB cells did not show any green fluorescence (Fig. **9**).

Some *in vitro* and *in vivo* studies indicated that the over-expression of the folate receptor on the tumor cell showed high affinity to the FA-dendrimer [66]. Several different groups researched FA-dendrimer conjugates in some different ways before. Several antitumor drugs like MTX and Doxorubicin were attached to FA-PAMAM dendrimer for the targeted delivery but more simple in structure and function. This multifunctional conjugate gives us an example of dendrimers extraordinary ability to functionalize their terminal groups with various targeting, therapeutic, and imaging agents.

3.3. Pass Through the Blood–Brain Barrier (BBB)

Costantino *et al*. [88] introduced a new method to prepare peptide- and *β*-D-glucose-covered dendrimer nanoparticles (NP). The NP has a poly (D,L-lactic-co-glycolic acid) (PLGA) core and a short peptide sequence modified surface (Fig. **11**). NP attached to short peptidic sequences in the *in vivo* experiment showed an ability to cross the blood–brain barrier after systemic administration (Fig. **12**).

In the *in vivo* experiment tetramethylrhodamine was used as label molecule. After femoral vein injecting, the brain slides of male albino rats showed red spots, which indicated that NP had successfully crossed the BBB and to penetrate into

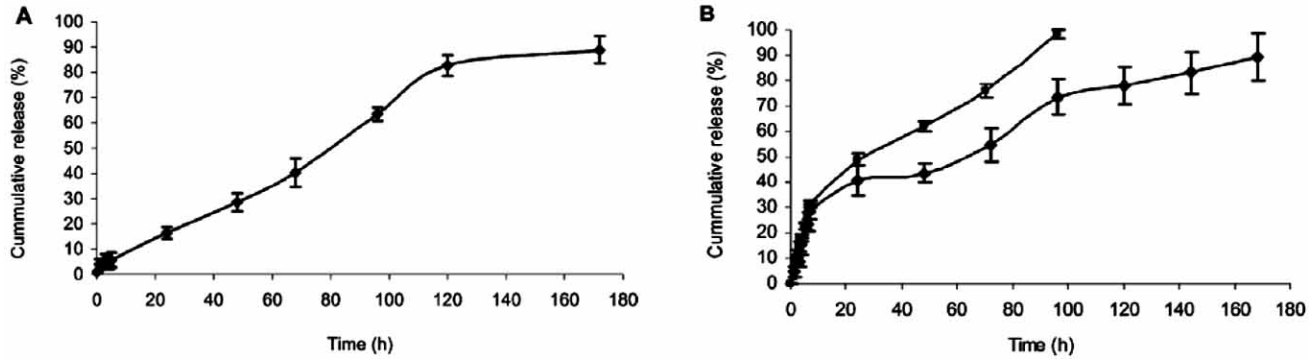


Fig. (8). Cumulative release profile of (A) rhodamine encapsulated in G2- dendrimer and (B) *β*-carotene encapsulated in G2-dendrimer (diamonds) and *β*-carotene suspension (squares). Data are mean ±SD.

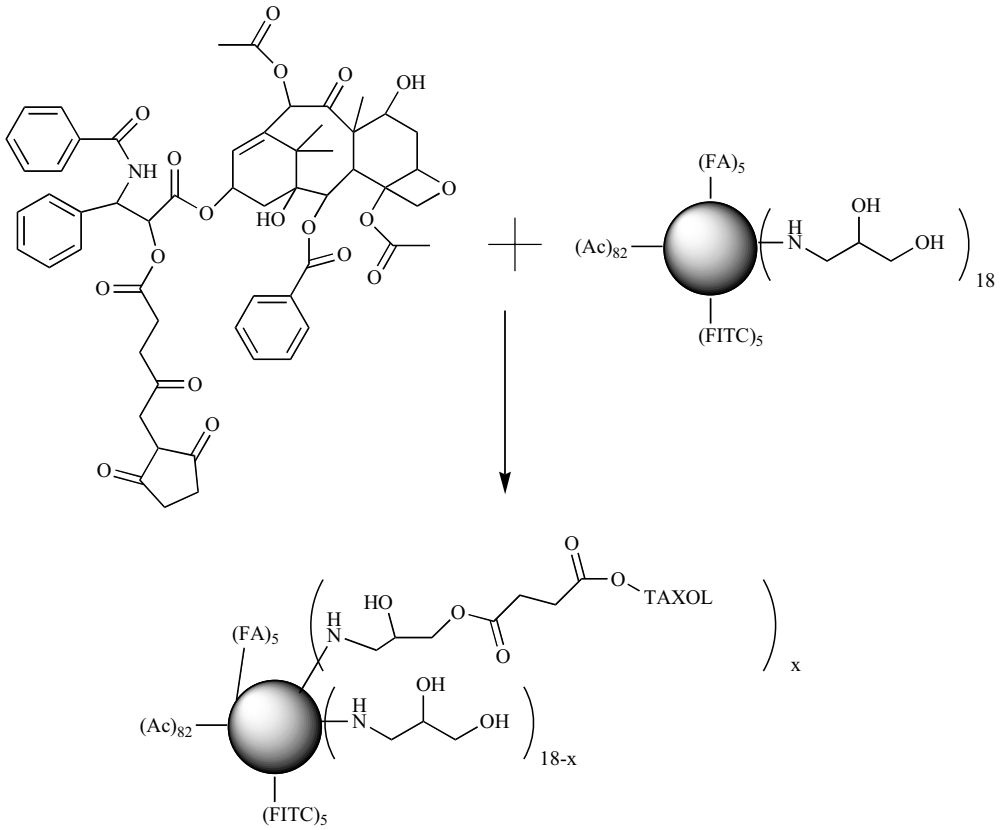


Fig. (9). Structure of conjugation of Taxol® to the carrier G5-Ac3-FITC-FA-OH, forming the trifunctional dendrimer conjugates G5-Ac3 (82)-FITC (5)-FA (5)-OH (15)-Taxol® (3).

the cells of the cerebral tissue after a systemic administration. The mechanism was not clear but the author believed that the NP was most likely functioned through endocytosis by the endothelial cells lining the brain blood capillaries.

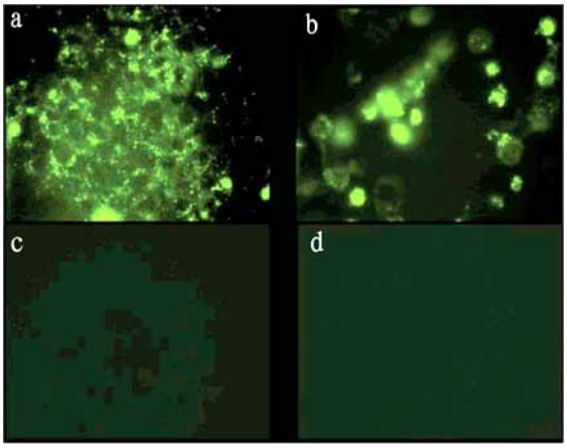
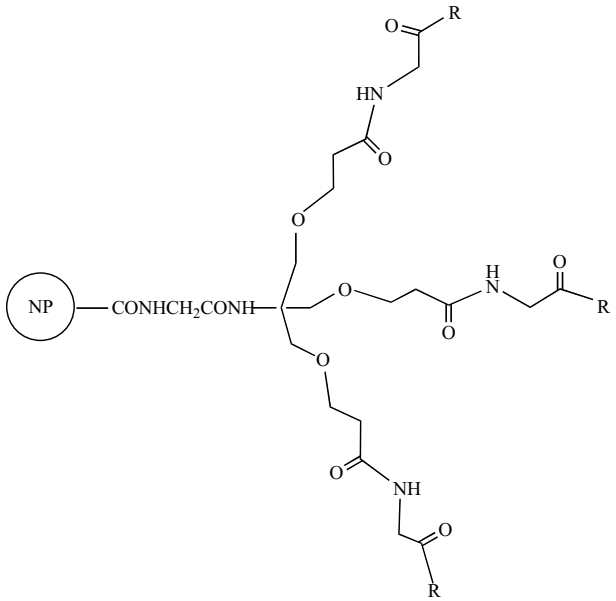


Fig. (10). KB(FR+) cells treated with either 100 nM G5-Ac3-FITC-FA (a) or 100 nM G5-Ac3-FITC-FA-OH-Taxol® (b) KB(FR-) cells treated with either 100 nM G5-Ac3-FITC-FA (c) or 100 nM G5-Ac3-FITC-FA-OH-Taxol® (d).

The PLGA core dendrimers were the first dendrimer known so far that has the ability to cross the BBB. It indicated that dendrimers could be used to carry drugs through the BBB into the brain for the disease of CNS (central nervous system).



(B) R=H_2N-Gly-Phe-DThr-Gly-Phe-Leu-Ser(O-β-D-glucose)-$CONH_2$

Fig. (11). The structure of the conjugate with a peptide surface. NP stands for the PLGA core.

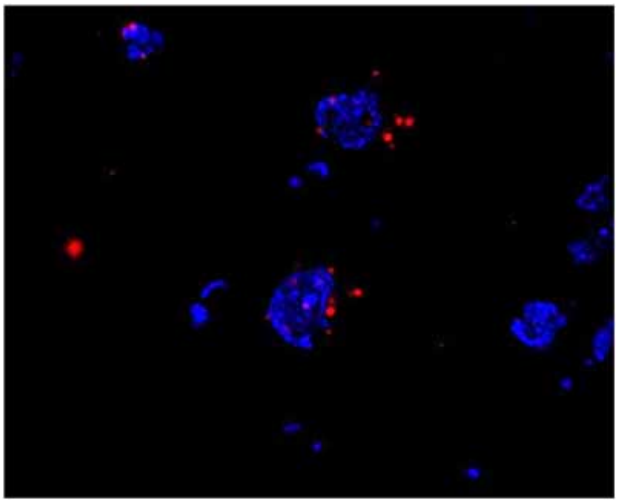


Fig. (12). Fluorescence microscopy images of representative rat brain slides. The presence of red spots, due to the labeling of NP by tetramethylrhodamine, indicates the presence of NP aggregates.

3.4. Drug-Dendrimer

Recently Tang *et al*.[89] synthesized a dendrimer (G1-G3) by the L-dopa (anti-Parkinson and depressant drug) (Fig. **13**). Individual L-dopa moieties in the dendrimer were connected to one another *via* hydrolysable diester linkages The L-dopa dendrimer showed a high solubility and high stability. In the past, as a prodrug being capable of passing the blood-brain barrier and becoming dopamine, the neurotransmitter, the main problem for L-dopa is the very low solubility and bioavailability. These properties restrict its use in the PD (parkinson disease) and would cause high toxicity.

Generation 2 of L-dopa containes 14 residues of L-dopa and generation 3 containes 30, which make up its core, branches, and periphery. We can imagine that when this dendrimer degrades, it may slowly release L-dopa in the circulation system or in the brain. Just a couple of mouths later, Tang group [90] reported another synthesis, the salicylate dendritic prodrugs (Fig. **14**).

The methods and the structures are similar to the previous one. Salicylic acid moieties are also connected to one another *via* hydrolysable diester linkages, and generation 3 salicylic acid dendrimer contains sixty salicylic acid residues. These researches give us a new idea that encapsulating by dendrimer is not the only way to enhance the solubility of non-soluble drugs; and they may also form dendrimers themselves, and gain some other advantages in controlled release.

CONCLUSIONS

In the past decade, especially recent years, applications of dendrimers in medical field have grown rapidly. The highly modifiable surface and architecture of these polymer provide a variety in structures and functions. The research of the formation process of dendrimer-DNA/RNA complexes indicates that the lower the formation energy is, the faster the formation process is. Dendrimer can be used as a carrier of drugs and DNA/RNA, a targeting vector, a fluorescence indicator and even as a photosensitizer in photochemical internalization. The recent researches showed that dendrimer could even construct by drugs with the specific end groups, just like L-dopa and salicylic acid.

However, dendrimers have a long way to go before they enter the clinical applications all the same. The toxicity, biocompatibility and the efficiency are three main barriers to

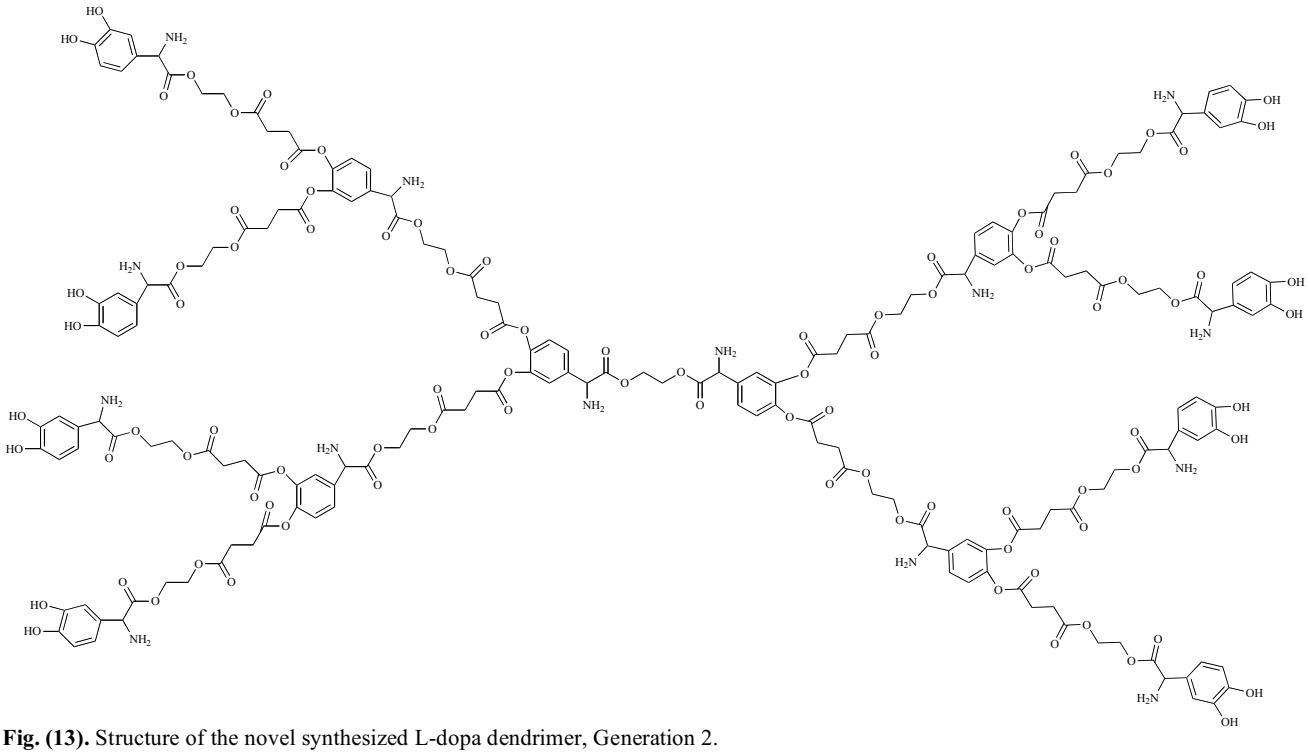


Fig. (13). Structure of the novel synthesized L-dopa dendrimer, Generation 2.

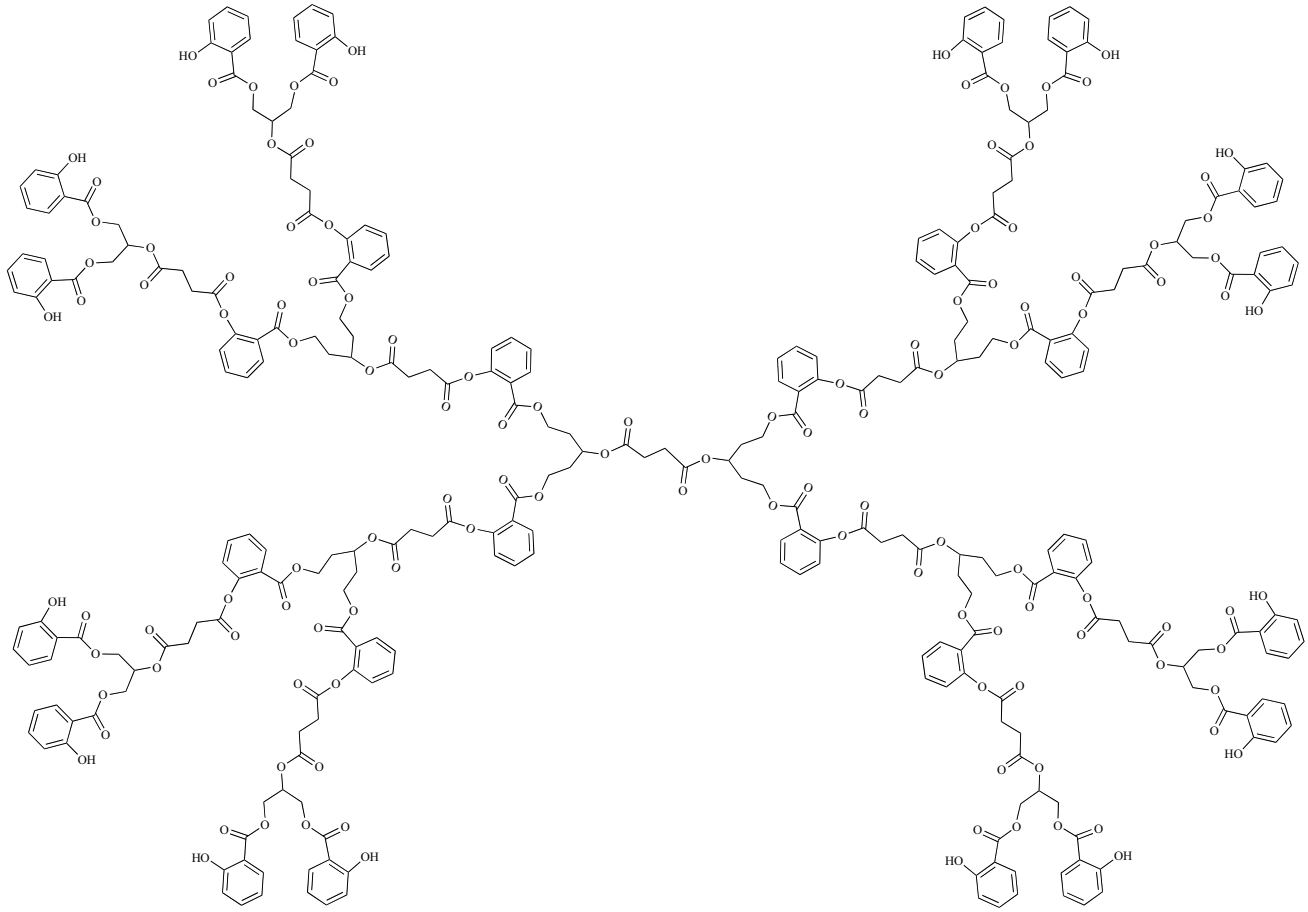


Fig. (14). Structure of a dendritic salicylic acid prodrug, generation 2.

these polymers. Although it is not easy to across these barriers, their ability to functionalize their terminal groups and structures gives us endless possibilities to solve all the problems. We have reasons to believe that dendrimers will have a bright future not only in medical applications due to their inherent advantages but also in gene delivery and drug delivery.

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REFERENCES

[1] Tomalia, D. A.; Baker, H.; Dewald, J. *Polym. J*., **1985**, *17*, 117.
[2] Frechét, J. M. J.; Tomalia, D. *Dendrimers and Other Dendritic Polymers*, Wiley, West Sussex, **2001**.
[3] Mazzola, L. *Nat. Biotechnol*., **2003**, *21*, 1137.
[4] Haensler, J.; Francis C. Szoka, J. *Bioconjug. Chem*., **1993**, *4*, 372.
[5] Tang, M. X.; Redemann, C. T.; Szoka, F. C. *Bioconjug. Chem*., **1996**, *7*, 703.
[6] Han, S.; Mahato, R. I.; Sung, Y. K.; Kim, S. W. *Mol. Ther.,* **2000**, *2*, 302.
[7] Zinselmeyer, B. H.; Mackay, S. P.; Schatzlein, A. G.; Uchegbu, I. F. *Pharm. Res.,* **2002**, *19*, 960.
[8] Tang, M. X.; Szoka, F. C. *Gene Ther.,* **1997**, *4*, 823.
[9] Svenson, S. *Curr. Opin. Colloid Interface Sci.,* **2004**, *9*, 201.
[10] Svenson, S. *J. Disper. Sci. Technol.,* **2004**, *25*, 101.
[11] Bielinska, A.; Kukowska-Latallo, J. F.; Johnson, J.; Tomalia, D. A.; Baker, J. R. *Nucleic Acids Res.,* **1996**, *24*, 2176.
[12] Kukowska-Latallo, J. F.; Bielinska, A. U.; Johnson, J.; Spindler, R.; Tomalia, D. A.; Baker, J. R. *P. Natl. Acad. Sci. USA,* **1996**, *93*, 4897.
[13] Patri, A. K.; Majoros, I. J.; Baker, J. R. *Curr. Opin. Chem. Biol.,* **2002**, *6*, 466.
[14] Newkome, G. R.; Moorefield, C. N.; Vogtle, F. *Dendrimers and Dendrons: Concepts, Syntheses, Applications*, Wiley-VCH, Weinheim, **2001**.
[15] Svenson, S.; Tomalia, D. A. *Adv. Drug Deliv. Rev.,* **2005**, *57*, 2106.
[16] Brown, M. D.; Schatzlein, A. G.; Uchegbu, I. F. *Int. J. Pharm.,* **2001**, *229*, 1.
[17] Dennig, J. In *Dendrimers V: Functional and Hyperbranched Building Blocks, Photophysical Properties, Applications in Materials and Life Sciences*; Shalley, C.A.; Vögtle, F., Eds.; Springer, Berlin, **2003**; pp. 227-236.
[18] Guo, C. Y.; Wang, H.; Lin, Y. H.; Cai, Q. L. *Prog. Biochem. Biophys.,* **2004**, *31*, 804.
[19] Ernst, N.; Ulrichskotter, S.; Schmalix, W. A.; Radler, J.; Galneder, R.; Mayer, E.; Gersting, S.; Plank, C.; Reinhardt, D.; Rosenecker, J. *J. Gene Med.,* **1999**, *1*, 331.
[20] Rittner, K.; Benavente, A.; Bompard-Sorlet, A.; Heitz, F.; Divita, G.; Brasseur, R.; Jacobs, E. *Mol. Ther.,* **2002**, *5*, 104.
[21] Luo, D.; Haverstick, K.; Belcheva, N.; Han, E.; Saltzman, W. M. *Macromolecules,* **2002**, *35*, 3456.
[22] Kim, T.; Seo, H. J.; Choi, J. S.; Jang, H. S.; Baek, J.; Kim, K.; Park, J. S. *Biomacromolecules,* **2004**, *5*, 2487.
[23] Schatzlein, A. G.; Zinselmeyer, B. H.; Elouzi, A.; Dufes, C.; Chim, Y. T. A.; Roberts, C. J.; Davies, M. C.; Munro, A.; Gray, A. I.; Uchegbu, I. F. *J. Control. Release,* **2005**, *101*, 247.
[24] Forrest, M. L.; Gabrielson, N.; Pack, D. W. *Biotechnol. Bioeng.,* **2005**, *89*, 416.

[25] Dufes, C.; Uchegbu, I. F.; Schatzlein, A. G. *Adv. Drug Deliver. Rev.,* **2005**, *57*, 2177.
[26] Bielinska, A. U.; Kukowska-Latallo, J. F.; Baker, J. R. *(BBA)-Gene Struct. Expr.,* **1997**, *1353*, 180.
[27] Abdelhady, H. G.; Allen, S.; Roberts, C. J.; Davies, M. C.; Tendler, S. J.; Williams, P. M. *Biophys. J.,* **2003**, *84*, 471.
[28] Ottaviani, M. F.; Sacchi, B.; Turro, N. J.; Chen, W.; Jockusch, S.; Tomalia, D. A. *Macromolecules,* **1999**, *32*, 2275.
[29] Choi, Y. S.; Cho, T. S.; Kim, J. M.; Han, S. W.; Kim, S. K. *Biophys. Chem.,* **2006**, *121*, 142.
[30] Okuda, T.; Kidoaki, S.; Ohsaki, M.; Koyama, Y.; Yoshikawa, K.; Niidome, T.; Aoyagi, H. *Org. Biomol. Chem.,* **2003**, *1*, 1270.
[31] Ottaviani, M. F.; Favuzza, P.; Sacchi, B.; Turro, N. J.; Jockusch, S.; Tomalia, D. A. *Langmuir,* **2002**, *18*, 2347.
[32] Plank, C.; Mechtler, K.; Szoka, F. C.; Wagner, E. *Hum. Gene Ther.,* **1996**, *7*, 1437.
[33] Zuhorn, I. S.; Kalicharan, R.; Hoekstra, D. *J. Biol. Chem.,* **2002**, *277*, 18021.
[34] Braun, C. S.; Vetro, J. A.; Tomalia, D. A.; Koe, G. S.; Koe, J. G.; Middaugh, C. R. *J. Pharm. Sci.,* **2005**, *94*, 423.
[35] Harada, A.; Kawamura, M.; Matsuo, T.; Takahashi, T.; Kono, K. *Bioconjug. Chem.,* **2006**, *17*, 3.
[36] Choi, J. S.; Joo, D. K.; Kim, C. H.; Kim, K.; Park, J. S. *J. Am.Chem. Soc.,* **2000**, *122*, 474.
[37] Choi, J. S.; Lee, E. J.; Choi, Y. H.; Jeong, Y. J.; Park, J. S. *Bioconjug. Chem.,* **1999**, *10*, 62.
[38] Aoi, K.; Motoda, A.; Ohno, M.; Tsutsumiuchi, K.; Okada, M.; Imae, T. *Polym. J.,* **1999**, *31*, 1071.
[39] Berg, K.; Selbo, P. K.; Prasmickaite, L.; Tjelle, T. E.; Sandvig, K.; Moan, D.; Gaudernack, G.; Fodstad, O.; Kjolsrud, S.; Anholt, H.; Rodal, G. H.; Rodal, S. K.; Hogset, A. *Cancer Res.,* **1999**, *59*, 1180.
[40] Nishiyama, N.; Arnida; Jang, W. D.; Date, K.; Miyata, K.; Kataoka, K. *J. Drug Target.,* **2006**, *14*, 413.
[41] Tack, F.; Bakker, A.; Maes, S.; Dekeyser, N.; Bruining, M.; Elissen-Roman, C.; Janicot, M.; Brewster, M.; Janssen, H. M.; De Waal, B. F. M.; Fransen, P. M.; Lou, X.; Meijer, E. W. *J. Drug Target.,* **2006**, *14*, 69.
[42] Van Rompaey, E.; Engelborghs, Y.; Sanders, N.; De Smedt, S. C.; Demeester, J. *Pharm. Res.,* **2001**, *18*, 928.
[43] Lee, J. H.; Lim, Y. B.; Choi, J. S.; Choi, M. U.; Yang, C. H.; Park, J. S. *B. Kor. Chem. Soc.,* **2003**, *24*, 1637.
[44] Al-Jamal, K. T.; Ruenraroengsak, P.; Hartell, N.; Florence, A. T. *J. Drug Target.,* **2006**, *14*, 405.
[45] Shah, D. S.; Sakthivel, T.; Toth, I.; Florence, A. T.; Wilderspin, A. F. *Int. J. Pharm.,* **2000**, *208*, 41.
[46] Ramaswamy, C.; Sakthivel, T.; Wilderspin, A. F.; Florence, A. T. *Int. J. Pharm.,* **2003**, *254*, 17.
[47] Kim, J. B.; Choi, J. S.; Nam, K.; Lee, M.; Park, J. S.; Lee, J. K. *J. Control. Release,* **2006**, *114*, 110.
[48] Hardy, J. G.; Kostiainen, M. A.; Smith, D. K.; Gabrielson, N. P.; Pack, D. W. *Bioconjug. Chem.,* **2006**, *17*, 172.
[49] Banerjee, P.; Weissleder, R.; Bogdanov, A. *Bioconjug. Chem.,* **2006**, *17*, 125.
[50] Kobayashi, H.; Kawamoto, S.; Bernardo, M.; Brechbiel, M. W.; Knopp, M. V.; Choyke, P. L. *J. Control. Release,* **2006**, *111*, 343.
[51] Kapp, T.; Dullin, A.; Gust, R. *J. Med. Chem.,* **2006**, *49*, 1182.
[52] Jimenez, R.; Garcia-Fernandez, E.; Sanchez, F. *Chem. Phys. Lett.,* **2006**, *420*, 372.
[53] Axel, D. I.; Spyridopoulos, I.; Riessen, R.; Runge, H.; Viebahn, R.; Karsch, K. R. *J. Vasc. Res.,* **2000**, *37*, 221.
[54] Jevprasesphant, R.; Penny, J.; Jalal, R.; Attwood, D.; McKeown, N. B.; D'Emanuele, A. *Int. J. Pharm.,* **2003**, *252*, 263.
[55] Liu, M. J.; Kono, K.; Frechet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.,* **1999**, *37*, 3492.
[56] Bhadra, D.; Bhadra, S.; Jain, N. K. *J. Pharm. Pharm. Sci.,* **2005**, *8*, 467.
[57] Namazi, H.; Adell, M. *Biomaterials,* **2005**, *26*, 1175.
[58] Yang, H.; Morris, J. J.; Lopina, S. T. *J. Colloid Interface Sci.,* **2004**, *273*, 148.
[59] Kojima, C.; Kono, K.; Maruyama, K.; Takagishi, T. *Bioconjug. Chem.,* **2000**, *11*, 910.
[60] Chen, H. T.; Neerman, M. F.; Parrish, A. R.; Simanek, E. E. *J. Am. Chem. Soc.,* **2004**, *126*, 10044.
[61] Bhadra, D.; Bhadra, S.; Jain, S.; Jain, N. K. *Int. J. Pharm.,* **2003**, *257*, 111.
[62] Kwon, G.; Naito, M.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. *J. Control. Release,* **1997**, *48*, 195.
[63] Yang, H.; Lopina, S. T. *J. Biomed. Mater. Res. A,* **2005**, *72A*, 107.
[64] Pasut, G.; Scaramuzza, S.; Schiavon, O.; Mendichi, R.; Veronese, F. M. *J. Bioact. Compat. Polym.,* **2005**, *20*, 213.
[65] Nguyen, P. M.; Hammond, P. T. *Langmuir,* **2006**, *22*, 7825.
[66] Wiener, E. C.; Konda, S.; Shadron, A.; Brechbiel, M.; Gansow, O. *Invest. Radiol.,* **1997**, *32*, 748.
[67] Kukowska-Latallo, J. F.; Candido, K. A.; Cao, Z. Y.; Nigavekar, S. S.; Majoros, I. J.; Thomas, T. P.; Balogh, L. P.; Khan, M. K.; Baker, J. R. *Cancer Res.,* **2005**, *65*, 5317.
[68] Shukla, S.; Wu, G.; Chatterjee, M.; Yang, W. L.; Sekido, M.; Diop, L. A.; Muller, R.; Sudimack, J. J.; Lee, R. J.; Barth, R. F.; Tjarks, W. *Bioconjug. Chem.,* **2003**, *14*, 158.
[69] Konda, S. D.; Aref, M.; Wang, S.; Brechbiel, M.; Wiener, E. C. *Magn. Reson. Mater. Phys. Biol. Med.,* **2001**, *12*, 104.
[70] Konda, S. D.; Wang, S.; Brechbiel, M.; Wiener, E. C. *Invest. Radiol.,* **2002**, *37*, 199.
[71] Kono, K.; Liu, M. J.; Frechet, J. M. J. *Bioconjug. Chem.,* **1999**, *10*, 1115.
[72] Patri, A. K.; Kukowska-Latallo, J. F.; Baker, J. R. *Adv. Drug Deliv. Rev.,* **2005**, *57*, 2203.
[73] Majoros, I. J.; Thomas, T. P.; Mehta, C. B.; Baker, J. R. *J. Med. Chem.,* **2005**, *48*, 5892.
[74] Quintana, A.; Raczka, E.; Piehler, L.; Lee, I.; Myc, A.; Majoros, I.; Patri, A. K.; Thomas, T.; Mule, J.; Baker, J. R. *Pharm. Res.,* **2002**, *19*, 1310.
[75] Wu, G.; Barth, R. F.; Yang, W. L.; Kawabata, S.; Zhang, L. W.; Green-Church, K. *Mol. Cancer Ther.,* **2006**, *5*, 52.
[76] Gurdag, S.; Khandare, J.; Stapels, S.; Matherly, L. H.; Kannan, R. M. *Bioconjug. Chem.,* **2006**, *17*, 275.
[77] Moon, W. K.; Lin, Y. H.; O'Loughlin, T.; Tang, Y.; Kim, D. E.; Weissleder, R.; Tung, C. H. *Bioconjug. Chem.,* **2003**, *14*, 539.
[78] Gillies, E. R.; Jonsson, T. B.; Frechet, J. M. J. *J. Am. Chem. Soc.,* **2004**, *126*, 11936.
[79] Hui, H.; Fan, X. D.; Cao, Z. L. *Polymer,* **2005**, *46*, 9514.
[80] Pistolis, G.; Malliaris, A.; Tsiourvas, D.; Paleos, C. M. *Chem. Eur. J.,* **1999**, *5*, 1440.
[81] You, Y. Z.; Hong, C. Y.; Pan, C. Y.; Wang, P. H. *Adv. Mater.,* **2004**, *16*, 1953.
[82] Haba, Y.; Harada, A.; Takagishi, T.; Kono, K. *J. Am. Chem. Soc.,* **2004**, *126*, 12760.
[83] Amir, R. J.; Shabat, D. *Chem. Commun.,* **2004**, 1614.
[84] Shamis, M.; Lode, H. N.; Shabat, D. *J. Am. Chem. Soc.,* **2004**, *126*, 1726.
[85] Dhanikula, R. S.; Hildgen, P. *Bioconjug. Chem.,* **2006**, *17*, 29.
[86] Cho, B. K.; Jain, A.; Nieberle, J.; Mahajan, S.; Wiesner, U.; Gruner, S. M.; Turk, S.; Rader, H. J. *Macromolecules,* **2004**, *37*, 4227.
[87] Majoros, I. J.; Myc, A.; Thomas, T.; Mehta, C. B.; Baker, J. R. *Biomacromolecules,* **2006**, *7*, 572.
[88] Costantino, L.; Gandolfi, F.; Bossy-Nobs, L.; Tosi, G.; Gurny, R.; Rivasi, F.; Angela Vandelli, M.; Forni, F. *Biomaterials,* **2006**, *27*, 4635.
[89] Tang, S. Z.; Martinez, L. J.; Sharma, A.; Chai, M. H. *Org. Lett.,* **2006**, *8*, 4421.
[90] Tang, S. Z.; June, S. M.; Howell, B. A.; Chai, M. H. *Tetrahedron Lett.,* **2006**, *47*, 7671.

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