molecular pharmaceutics

reviews

Molecular Engineering of Dendritic Polymers and Their Application as Drug and Gene Delivery Systems

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Received July 25, 2006; Revised Manuscript Received November 9, 2006; Accepted November 29, 2006

Abstract: This review discusses the development of functional and multifunctional dendrimeric and hyperbranched polymers, collectively called dendritic polymers, with the objective of being applied as drug and gene delivery systems. In particular, using as starting materials known and well-characterized basic dendritic polymers, the review deals with the type of structural modifications to which these dendritic polymers were subjected for the development of drug carriers with low toxicity, high encapsulating capacity, a specificity for certain biological cells, and the ability to be transported through their membranes. Proceeding from functional to multifunctional dendritic polymers, one is able to prepare products that fulfill one or more of these requirements, which an effective drug carrier should exhibit. A common feature of the dendritic polymers is the exhibition of polyvalent interactions, while for multifunctional derivatives, a number of targeting ligands determine specificity, another type of group secures stability in biological milieu and prolonged circulation, while others facilitate their transport through cell membranes. Furthermore, dendritic polymers employed for gene delivery should be or become cationic in the biological environment for the formation of complexes with the negatively charged genetic material.

Keywords: Dendrimers; hyperbranched polymers; dendritic polymers; nanocarriers; drug delivery system; gene delivery

1. Introduction

Dendrimers are highly branched and monodisperse macromolecules with symmetrical, nanometer-sized architecture which are prepared by multistep synthetic procedures.[1−5] They consist of a central core, branching units, and terminal functional groups. This type of architecture induces the formation of nanocavities, the environment of which determines their solubilizing or encapsulating properties, while the external groups primarily characterize their solubility and chemical behavior. Hyperbranched polymers[6−8] are also branched and exhibit nanocavities, but in contrast to dendrimers, they are nonsymmetrical and polydispersed. In addition to these properties, the latter polymers, including the extensively investigated hyperbranched polyether polyols

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10.1021/mp060076n CCC: $37.00
Published on Web 01/13/2007

or polyglycerols,[9−15] are conveniently prepared, and therefore, they are less expensive compared to dendrimers, which are prepared under tedious multistep reaction schemes.

Dendrimeric and hyperbranched polymers are called collectively dendritic polymers, the nanocavities of which can encapsulate various molecules, including active drug ingredients. The external groups of dendritic polymers can be modified, providing a diversity of functional materials[16] that are employed for various applications, including drug delivery. Thus, commercially available or custom-made dendrimeric or hyperbranched polymers have been functionalized and are used as drug delivery systems,[17−24] as gene delivery vectors,[25−28] or as drugs on their own.[29] Due to the presence of several terminal groups at the surface of dendritic polymers, it is possible, through appropriate functionalization, that some of them can be modified to one type of functional group while others to another type, leading finally to the preparation of the so-called multifunctional dendritic polymers. A schematic example is shown in Figure 1. Each type of external groups plays a specific function when these multifunctional dendritic polymers are applied as drug delivery systems. For instance, specificity for certain cells has been achieved by attaching targeting ligands at the surface of dendritic polymers, the transport through cell membranes by groups inducing translocation, while enhanced solubility, decreased toxicity, biocompatibility, stability, and protection in the biological milieu have been achieved by functionalizing the terminal groups of dendritic polymers with poly(ethylene glycol) (PEG) chains. The function of

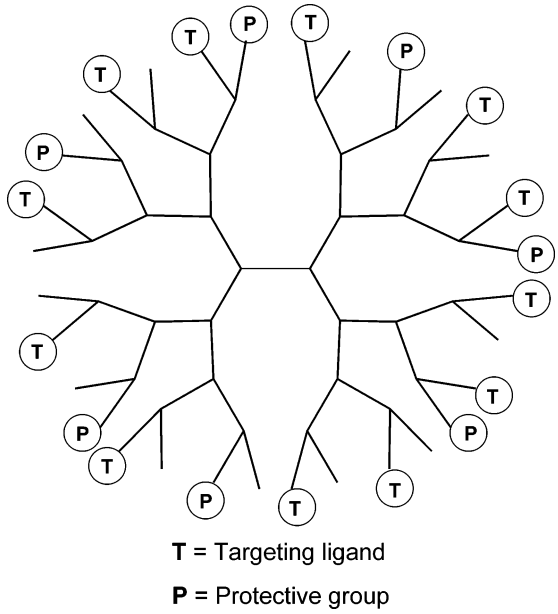

Figure 1. Schematic representation of a multifunctional dendrimer.

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PEG chains is crucial for modifying the behavior of drugs themselves or that of drug carriers.[30−38]

In this regard, it should be noted that the modifications of the surface groups of dendritic polymers as mentioned above had been previously applied to liposomes. In fact, liposomes bearing at their surface targeting ligands[39,40] which are complementary to cell receptors and also protective groups which prolong their circulation in biological fluids have been developed.[41−44] The latter property was almost exclusively achieved by coating the surface of the liposomes with PEG chains, yielding PEGylated liposomes also called stealth liposomes. Another crucial parameter for obtaining effective drug delivery systems is their transport through cell membranes which was achieved by the introduction of translocating agents at the liposomes' external surface.[45,46]

Targeting ligands which are complementary to cell receptors lead to the attachment of the dendritic nanocarrier to the cell surface. This binding is further induced or facilitated by polyvalent interactions[47−49] attributed to the proximity of the recognizable ligands at the dendritic surface. Furthermore, as already mentioned, PEG chains increase the stability of liposomes, prolonging their circulation, and therefore, analogous behavior should be expected for PEGylated dendritic polymers. Transport through the cell membrane can, in principle, be facilitated by the introduction of molecular transporters at the surface of the dendritic polymers. On the other hand, modification of the internal groups of dendrimers affects their solubilizing character, rendering possible the encapsulation of a diversity of drugs. Finally, cationization of dendrimers induces the interaction with DNA for the formation of DNA−dendritic polymer complexes employed in gene therapy.

Monofunctional dendritic drug carriers cannot simultaneously exhibit the properties mentioned above which characterize the multifunctional derivatives. It is the purpose of this review, starting from selected and primarily commercially available monofunctional dendritic polymers, to molecular engineer their surface to produce multifunctional systems which will be employed for drug delivery and gene transfection. Such dendritic polymers are the poly(amidoamine) (PAMAM), diaminobutane poly(propylene imine) (DAB), and also the polyglycerol (PG) and poly(ethylene imine) (PEI) hyperbranched polymers (Chart 1). This review is by no means exhaustive, and only selected examples will be presented, highlighting dendritic polymers for which drug delivery properties are modified when their surfaces are modified through molecular engineering. It is exactly the objective of this review to illustrate the effectiveness of surface modification of dendritic polymers to prepare drug carriers with desired properties. The influence each type of group exerts on the behavior of functional dendritic polymers and specifically on solubility, biological stability, biocompatibility and toxicity, targeting, and transport properties will be presented. Many in vitro studies demonstrate the great

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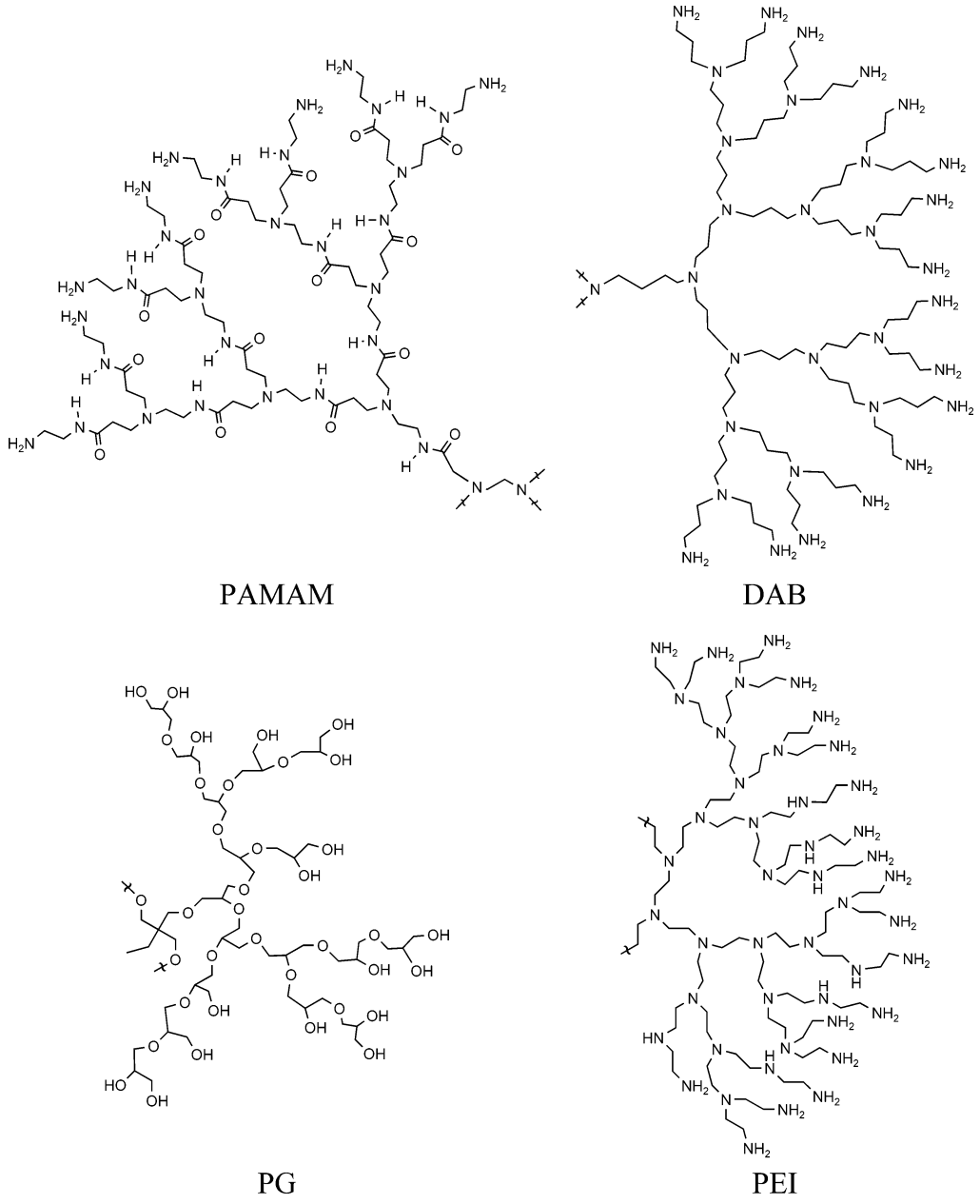
Chart 1



potential of dendritic polymers as drug delivery systems, but relatively few in vivo studies have been reported. This topic has very recently been reviewed.[50]

2. From Mono- to Multifunctional Dendrimeric Polymers as Drug Carriers

Employing as a starting polymer the most widely employed PAMAM dendrimer and applying a molecular engineering strategy on its terminal primary amino groups, poly(ethylene glycol) monomethyl ether (M-PEG) chains, with an average molecular weight of 550 or 2000, were introduced on the surface of the third-generation (PAMAM-G3) and fourth-generation (PAMAM-G4) dendrimers, as shown in Figure 2. Inside the nanocavities of the so-prepared PEGylated dendrimers, adriamycin (ADR) or methotrexate (MTX), anticancer drugs were encapsulated.[51]

As the amount of ADR employed in the encapsulation experiments increased, the number of ADR molecules solubilized in the dendrimer also increased and finally reached a plateau. Depending on the dendrimeric derivative generation, i.e., M-PEG(550)-PAMAM-G3, M-PEG(2000)-

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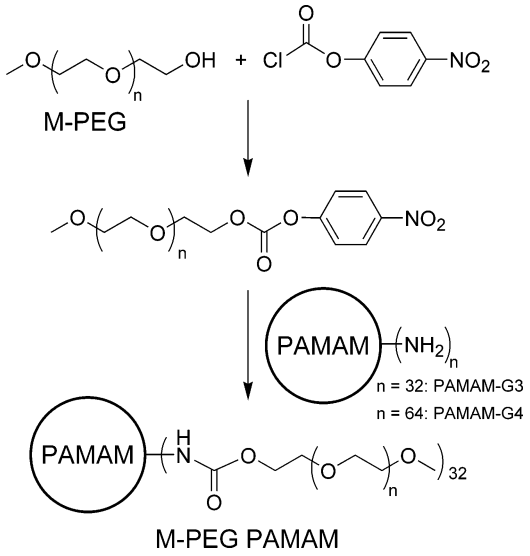
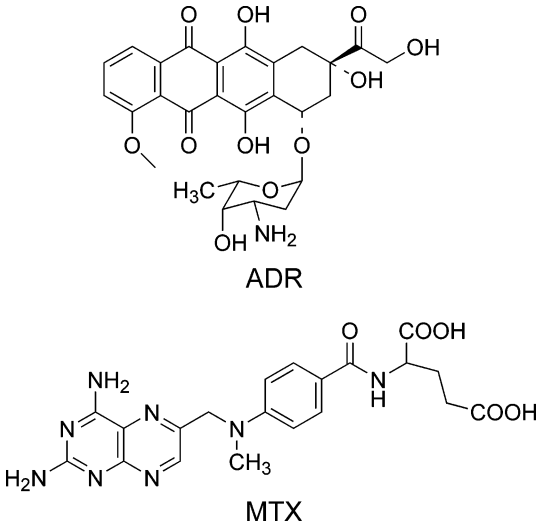


Figure 2. Preparation and structure of M-PEG PAMAM dendrimer of the third and fourth generation.



PAMAM-G3, M-PEG(550)-PAMAM-G4, or M-PEG(2000)-PAMAM-G4, the maximum number of ADR molecules encapsulated per dendrimer was ca. 1.2, 2.3, 1.6, or 6.5, respectively (Figure 3). It is apparent that the encapsulation ability varied for these PEGylated dendrimers and was found to depend on the molecular weight of PEG moieties and also on the generation of dendrimers.

The acidic MTX, bearing two carboxyl groups, can effectively be encapsulated inside the basic interior of PAMAM. The number of loaded MTX molecules per dendrimeric derivative expressed as the MTX/dendrimer ratio is shown in Figure 4. As in the case of ADR encapsulation, the number of encapsulated MTX molecules increased as the amount of MTX increased during loading and finally reached a constant value. In this case, the maximum number of MTX molecules encapsulated in M-PEG(550)-PAMAM-G3, M-PEG(2000)-PAMAM-G3, M-PEG(550)-PAMAM-G4, or M-PEG(2000)-PAMAM-G4 dendrimers was approximately 10, 13, 20, or 26 mol/mol of dendrimer, respectively. Thus, the number of molecules encapsulated by the PEGylated dendrimers increased when MTX was applied instead of ADR. Since these two drugs have almost similar molecular weights, the enhanced encapsulation of MTX was attributed to the electrostatic interactions. In a manner analogous to ADR encapsulation, the number of MTX encapsulated inside the dendrimer was affected both by the generation of PAMAM and by the chain length of the M-PEG.

Release experiments performed in PBS (phosphate-buffered saline) showed that ADR was readily released from the modified dendrimers. Apparently, hydrophobic interac-

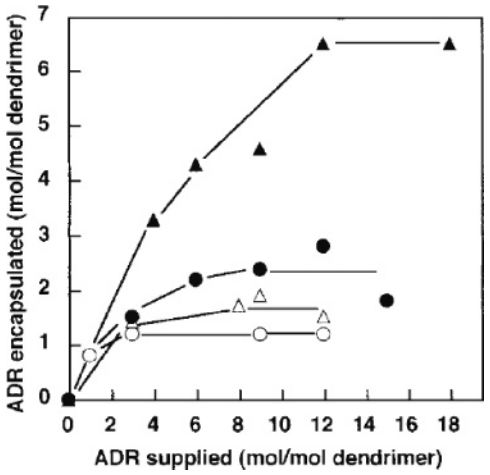


Figure 3. Encapsulation of ADR by M-PEG(550)-attached (○ and △) or M-PEG(2000)-attached (● and ▲) PAMAM-G3 (○ and ●) and PAMAM-G4 (△ and ▲) dendrimers. The number of ADR molecules encapsulated per dendrimer is shown as a function of the ADR:dendrimer molar ratio during loading. Reprinted from ref 51. Copyright 2000 American Chemical Society.

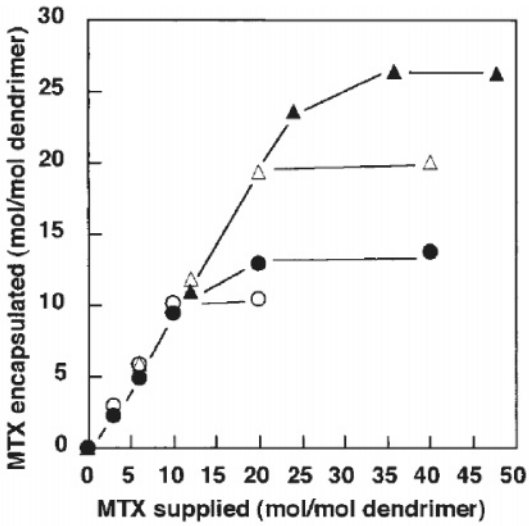


Figure 4. Encapsulation of MTX by M-PEG(550)-attached (○ and △) or M-PEG(2000)-attached (● and ▲) PAMAM-G3 (○ and ●) and PAMAM-G4 (△ and ▲) dendrimers. The number of MTX molecules encapsulated per dendrimer is shown as a function of the MTX:dendrimer molar ratio during loading. Reprinted from ref 51. Copyright 2000 American Chemical Society.

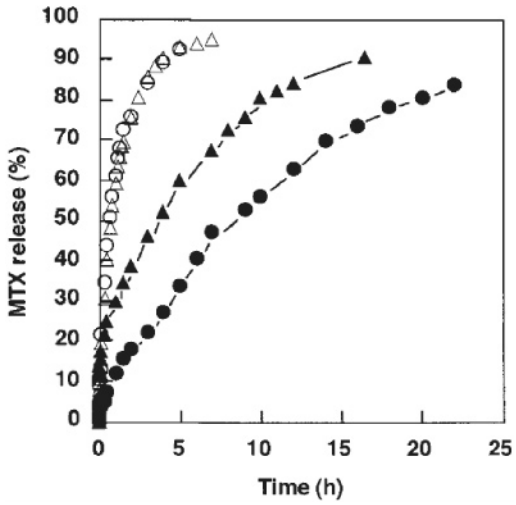


Figure 5. Release of MTX from the M-PEG(2000)-attached PAMAM-G4 dendrimer. The MTX-loaded M-PEG(2000)-PAMAM-G4 dendrimer (○ and ●) or free MTX (△ and ▲) was dissolved in 1 mM Tris-HCl-buffered solution (pH 7.4) with (○ and △) or without (● and ▲) 150 mM NaCl and dialyzed against the same solution. The time course of MTX concentration in the outer phase during the dialysis is shown. Reprinted from ref 51. Copyright 2000 American Chemical Society.

tion between ADR and the dendrimer is not sufficiently strong to keep the drug in the interior of the PAMAM nanocavities. Release of MTX from the M-PEG-functionalized dendrimers was also investigated by the same method. In dialysis experiments, the concentration of MTX in the outer phase as a function of time is shown in Figure 5. It is clear that the MTX concentration in the outer phase increased at a slower pace when MTX was encapsulated in the M-PEG-attached dendrimer compared to nonencapsulated free MTX. This indicates that MTX was gradually released from the modified dendrimer. Since MTX was electrostatically bound to the dendrimeric interior, its release was suppressed to some extent. However, when the dialysis was performed in the presence of 150 mM NaCl, a difference was not observed in the rate of release between MTX encapsulated in the PEGylated dendrimer and free MTX. In the presence of the salt, MTX can dissociate readily from the dendrimer because the electrostatic interaction is weakened by the shielding effect of Na^+ and Cl^-.[51]

Encapsulation of hydrophobic drugs was also achieved employing a PEGylated diaminobutane poly(propylene imine) dendrimer of the fifth generation (DAB64).[52] PEGylation of dendrimers was performed under facile experimental conditions by the interaction of methoxy poly(ethylene glycol) isocyanate (M-PEG-isocyanate; MW = 5000 g/mol) with the terminal primary amino groups of DAB64, as shown in Figure 6. Two different PEGylated dendrimeric derivatives were prepared, i.e., DAB64-4PEG (weakly PEGylated) and

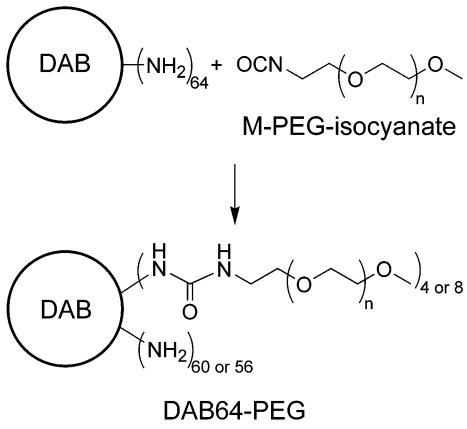
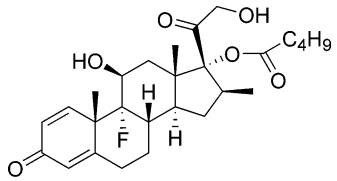


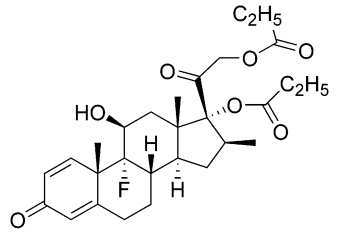
Figure 6. Preparation of the PEGylated DAB dendrimer of the fifth generation functionalized with four or eight PEG chains.

DAB64-8PEG (densely PEGylated). In this manner, the role of PEG coating in encapsulation and release properties was assessed.

The encapsulating ability of the parent and PEGylated DAB derivatives was assessed by employing betamethasone valerate (BV) and betamethasone dipropionate (BD).



BV



BD

These anti-inflammatory corticosteroids are practically water insoluble, and it is, therefore, necessary to encapsulate these compounds in a water-soluble carrier to facilitate their use as drugs. As shown in Table 1, the concentration of encapsulated betamethasone derivatives was significantly increased in PEGylated dendrimers. Thus, for DAB64-8PEG, the loading was 13 and 7 wt % for BV and BD, respectively, while for DAB64-4PEG, the loading was 6 and 4 wt %, respectively. The enhanced solubility increase was attributed to an additional solubilization of the compounds in PEG chains with which the dendrimers are coated (Figure 7). This is also verified by the fact that upon protonation they remain solubilized in the environment of PEG chains.

To establish the exact site of solubilization and release properties of these PEGylated dendrimers, the hydrophobic pyrene was employed. This is a well-known and very sensitive probe, which is used as a model compound when

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Table 1. Comparative Solubility of Pyrene (PY), Betamethasone Valerate (BV), and Betamethasone Dipropionate (BD) in Parent DAB64 Dendrimers and in Their PEGylated Derivatives[a]

compound	[dendrimer] (M)	[PY] (M)	[BV] (M)	[BD] (M)
DAB64	5×10^{-5}	2.15×10^{-6}	2.95×10^{-5}	1.84×10^{-5}
DAB64-8PEG	5×10^{-5}	5.40×10^{-5}	3.85×10^{-4}	2.56×10^{-4}
DAB64-4PEG	5×10^{-5}	2.14×10^{-5}	2.05×10^{-4}	1.25×10^{-4}
DAB64-8PEG	5×10^{-4}	8.75×10^{-5}	3.65×10^{-3}	1.87×10^{-3}
DAB64-4PEG	5×10^{-4}	5.25×10^{-5}	1.70×10^{-3}	1.09×10^{-3}

[a] Taken with permission from ref 52. Copyright 2001 Elsevier B.V.

drugs cannot provide this type of information. Employing the I_1/I_3 fluorescence intensity ratio, which probes the polarity of the medium,[53] it was found that hydrophobic pyrene is solubilized both in the core and in PEG chains. Furthermore, when loaded PEGylated dendrimers were protonated, pyrene is not released in the bulk aqueous phase as judged by the I_1/I_3 ratio and fluorescence intensity (F/F^0) values. This was attributed to the fact that as pyrene is leaving the core due to protonation it is solubilized inside PEG chains. According to the I_1/I_3 fluorescence intensity ratio, pyrene is solubilized neither in the bulk water phase nor in the interior of the dendrimer. If the PEG chains were not present, one would expect release of pyrene in water, since the environment of the nanocavities becomes polar due to protonation, and therefore, the hydrophobic pyrene cannot remain solubilized. In addition, protonated tertiary amino groups of the core do not exhibit the ability to form charge transfer complexes with pyrene,[54] and therefore, encapsulation of the pyrene is not induced. It should, however, be noted that complete release of pyrene can be achieved upon exhaustive dilution of the PEGylated dendrimer. The same behavior was observed for the hydrophobic drugs BV and BD. In conclusion, the enhanced solubilization of these drugs in PEGylated dendrimers secures their application as promising controlled release drug delivery systems.

Extending the previous work, Paleos et al. prepared a novel multifunctional dendrimeric carrier based also on DAB64.[55] The synthetic steps for obtaining this multifunctional carrier are shown in Figure 8. This carrier is intended to simultaneously address issues such as stability and prolonged circulation in the biological milieu, the solubility increase, targeting, and very possibly transport through cell membranes. For this purpose, in addition to surface protective poly(ethylene glycol) chains, guanidinium moieties were introduced as targeting ligands. This accumulation of guanidinium groups at the surface of the dendrimer may also facilitate its transport in a manner analogous to that of oligoarginine peptides.[56,57] This dendrimeric derivative due to the type of selected functional groups can, in principle, exhibit multifunctional drug delivery properties such as (a) protection of the carrier because of its coverage with poly(ethylene glycol) chains, (b) targeting ability toward complementary moieties (guanidinium groups secure the facile interaction with acidic receptors, including the biologically significant carboxylate and phosphate groups), (c) the possibility of encapsulation and release of active drug ingredients from the nanocavities, which can be tuned by medium changes,[52] (d) complexation ability with negatively charged DNA for gene therapy applications, (e) the occurrence of polyvalent interactions, which are associated with enhanced binding, due to the accumulation of recognizable moieties on the limited surface area of the dendrimer, and (f) the toxicity decrease anticipated by the facile modification of the toxic amino groups.[58]

Loading capacity and release properties of this multifunctional dendrimer were tested with betamethasone valerate (BV) and pyrene (Py) model compounds. The multifunctional dendrimeric derivative encapsulated significantly higher concentrations of the compounds mentioned above compared to the parent dendrimer as determined by UV spectroscopy (Table 2). This is particularly useful for the hydrophobic betamethasone valerate, of which seven molecules are solubilized per dendrimeric molecule. Specifically, for betamethasone valerate, the loading capacity is 11 wt % inside the multifunctional dendrimer, i.e., almost double compared to the loading capacity of the simply PEGylated dendrimer (6 wt %)[52] and more than 5 times greater compared to the loading capacity of the parent dendrimeric solution (1.7 wt %).

The release of the active ingredient from the dendrimer when it reaches the target site enhances its bioavailability and efficacy. In addition, the release of drug from the endosomal compartment appears to be a limiting factor for several targeted drug delivery formulations. These requirements impose the need for the development of drug delivery systems in which the release of drug can be triggered by an appropriate stimulus. For this purpose, pH-triggered, enzymatic, thermal, and photochemically induced processes have

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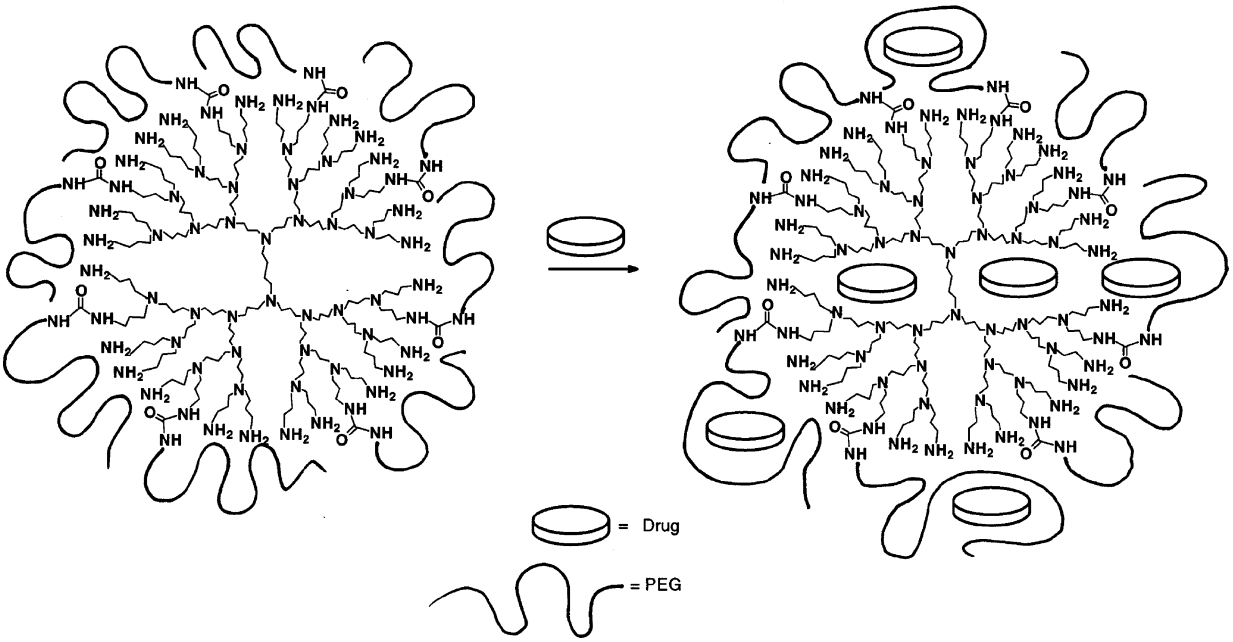

Figure 7. Schematic representation of the solubilization of a drug in PEGylated DAB dendrimers. Reprinted with permission from ref 52. Copyright 2001 Elsevier B.V.

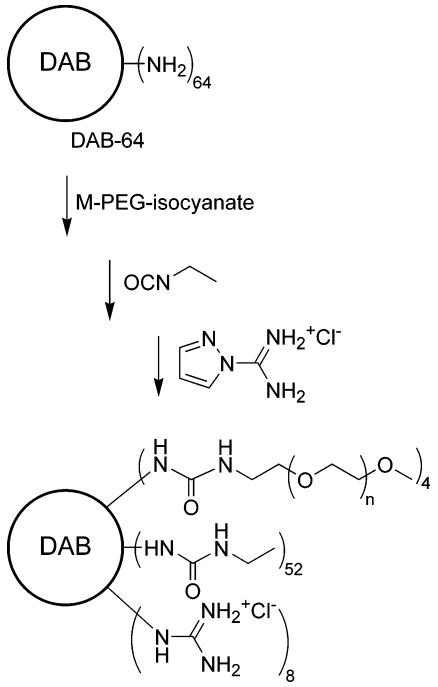

Figure 8. Reaction scheme for the synthesis of a multifunctional dendrimeric derivative.

been reported.[59] For instance, a low pH within endosomal and ischemic tissue environments renders acid triggerable delivery systems attractive for controlled release. The previously prepared multifunctional poly(propylene imine) dendrimers, due to the presence of tertiary amino groups in their interior, fulfill at least one of these requirements; i.e., they are pH-responsive. Due to the pH response of the interior of these dendrimers, pyrene or any other hydrophobic molecule solubilized in their interior is relocated to the PEG coating upon protonation of the tertiary amines of the nanocavities. Since acidification proved to be noneffective in releasing the encapsulated pyrene model compound from the PEG protective coating, another method has been investigated. Thus, an aqueous sodium chloride solution has been applied for triggering pyrene release since, as established in independent studies,[60,61] alkali metal cations form complexes with ether moieties of poly(ethylene glycol) chains. The so-prepared dendrimeric derivative, due to the attachment of PEG chains at its surface, can be susceptible to analogous interactions, and therefore, the sodium cation can replace the solubilized pyrene probe, releasing it to the bulk aqueous phase. In fact, via titration of dendrimeric solutions with a sodium chloride solution, pyrene was released and dispersed in the bulk aqueous phase in the form of crystallites.

This two-step triggered release from the multifunctional dendrimer was also applied for the lipophilic drug BV. Release of the drug with hydrochloric acid has not been found since BV remained solubilized within the dendrimeric

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Table 2. Comparative Solubility of Pyrene (PY) and Betamethasone Valerate (BV) in the Parent DAB64 and Multifunctional Dendrimer[a]

compound	[dendrimer] (M)	[PY] (M)	PY:dendrimer molar ratio	[BV] (M)	BV:dendrimer molar ratio
DAB64	1.0×10^{-3}	$(2.1 \pm 0.2) \times 10^{-5}$	0.021 ± 0.002	$(2.5 \pm 0.4) \times 10^{-4}$	0.25 ± 0.04
multifunctional dendrimer	2.5×10^{-4}	$(1.9 \pm 0.08) \times 10^{-5}$	0.076 ± 0.002	$(1.80 \pm 0.4) \times 10^{-3}$	7.20 ± 0.03

[a] Taken from ref 55. Copyright 2004 American Chemical Society.

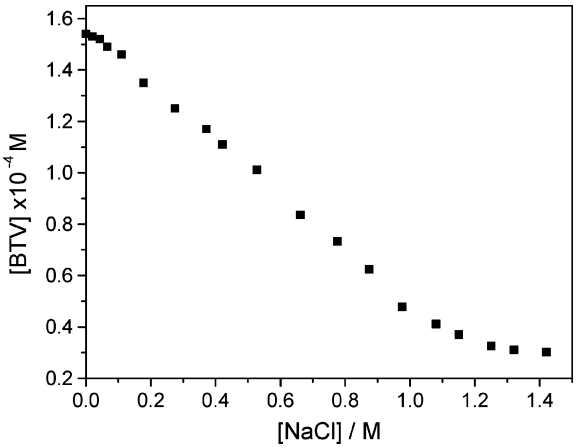

Figure 9. Plot of the betamethasone valerate concentration in a 2.50×10^{-5} M dendrimeric solution as a function of the amount of added NaCl. Reprinted from ref 55. Copyright 2004 American Chemical Society.

environment and preferably within the poly(ethylene glycol) chains. However, BV encapsulated in this dendrimeric derivative was completely released upon addition of sodium chloride as shown in Figure 9. It is interesting, however, to note that within the concentration range of the sodium cation present in extracellular fluids, i.e., 0.142 M,[62] the BV was released in relatively small quantities. The betamethasone valerate gradually released from the multifunctional dendrimer formed crystallites as identified by ^{1}H NMR.

It is therefore advisable to follow the fate of the bioactive compound when this is encapsulated in PEGylated dendrimers in experiments in vitro and before it is applied in experiments in vivo, since sodium chloride in extracellular fluids and potassium chloride in the intracellular environment can be complexed with PEG chains, affecting the overall release profile of the drug. Thus, the possibility of triggering premature drug release in the extracellular fluid, i.e., before endocytosis to the target cells, should be taken into account when designing a targeted PEGylated drug delivery system.

Before the application of multifunctional dendrimers to cells, their drug delivery efficiency was assessed and modeled by investigating their interaction with multilamellar liposomes consisting of phosphatidylcholine, cholesterol, and dihexadecyl phosphate (19:9.5:1) and dispersed in aqueous or phosphate buffer solutions.[63] In fact, liposomes are considered the closest analogue of cells. These multilamellar liposomes bear the phosphate moiety as the complementary group of the guanidinium located at the surface of the dendrimer. In these experiments, the poly(propylene imine) dendrimer of the fourth generation was functionalized with six (DAB-G6) or 12 (DAB-G12) guanidinium groups as targeting ligands, while the remaining toxic external primary amino groups of the dendrimers were allowed to interact with propylene oxide, affording the corresponding hydroxylated derivatives. The reaction scheme modifying the dendrimeric surface is shown in Figure 10. The DAB-G0 dendrimer does not contain any guanidinium groups, and it is used as a reference compound. The so-prepared dendrimers were loaded with corticosteroid drugs, i.e., BV and BD for investigating their transfer to liposomes.

Liposomes are molecularly recognized by dendrimers which act as a kind of "molecular glue" adhering liposomes with each other and leading to the formation of large aggregates at dendrimer:dihexadecyl phosphate molar ratios higher than 1:30, as observed with phase contrast optical microscopy. Calcein liposomal entrapment experiments demonstrate a limited leakage, which is less than 13%, following liposomal interaction with the guanidinylated dendrimers at a 1:25 dendrimer:DHP molar ratio. The latter experiment indicates that the membrane of the liposomes remains almost intact during their molecular recognition with these dendrimers. The enthalpy of the interaction is dependent on the number of guanidinium groups present at the dendrimeric surface. This behavior is further confirmed by the fact that the process of liposome−dendrimer adhesion is reversed and redispersion of the aggregates occurs with the addition of concentrated phosphate buffer.

The interaction between these drug-loaded dendrimers and multilamellar liposomes results in the transport of drugs from the dendrimeric derivatives to the "empty" liposomes as summarized in Table 3. The experiments demonstrate that ∼25% of BD or BV is present in the precipitated aggregates when DAB-G0 is used. When the targeting guanidinylated dendrimers DAB-G6 and DAB-G12 were used, the amount of drug in the precipitate increases substantially, becoming ∼60 and ∼80%, respectively. The functionalization with guanidinium groups at the external surface of the dendrimers results in an effective adhesion to the multilamellar lipo-

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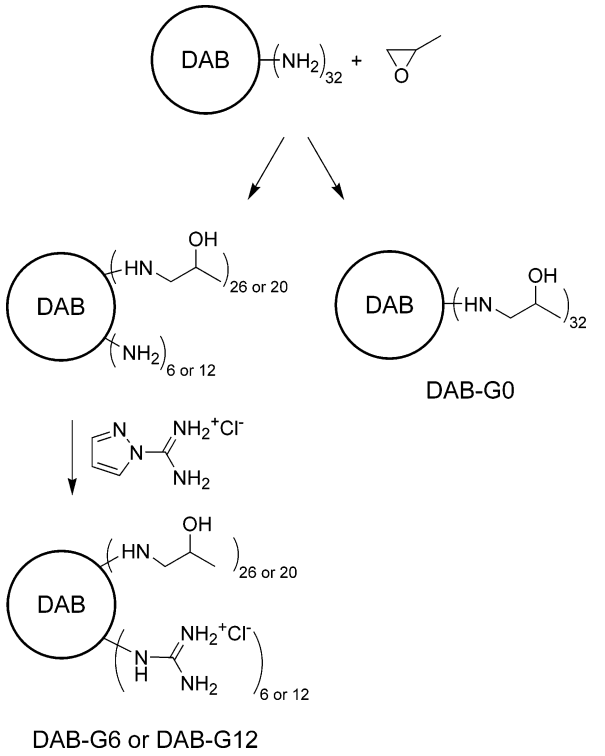


Figure 10. Functionalization of the poly(propylene imine) dendrimer of the fourth generation, including guanidinylation at the final step.

Table 3. Drug Transfer (%) from Drug-Loaded Dendrimers to Multilamellar Liposomes in Aggregates Obtained after Their Interaction in Water or in 10 mM Phosphate Buffer (pH 7.4) and in Multilamellar Liposomes Obtained following Redispersion of the Aggregates[a]

		drug transfer (%) in aggregates		drug transfer (%) after redispersion	
drug	dendrimer	water	phosphate buffer	water	phosphate buffer
BD	DAB-G0	24.4 ± 2.4	19.8 ± 1.2	15.8 ± 0.9	12.1 ± 1.1
	DAB-G6	62.5 ± 1.9	48.5 ± 1.6	28.1 ± 1.7	24.5 ± 1.3
	DAB-G12	84.5 ± 2.1	68.4 ± 1.5	45.1 ± 1.8	40.0 ± 1.4
BV	DAB-G0	32.9 ± 2.0	27.1 ± 1.0	15.9 ± 1.2	14.1 ± 0.9
	DAB-G6	59.0 ± 1.5	39.5 ± 2.1	29.0 ± 1.0	26.1 ± 1.5
	DAB-G12	78.1 ± 2.3	57.5 ± 2.0	42.0 ± 1.5	38.2 ± 1.2

[a] Taken from ref 63. Copyright 2005 American Chemical Society.

somes. In experiments taking place in 10 mM phosphate buffer, the amount of drug present in the aggregates decreases slightly. This decrease in drug transport can be rationalized by the competitive interaction of the phosphate groups in bulk with the guanidinium dendrimeric groups leading to less effective adhesion with the multilamellar liposomes.

Carbohydrates which are, in general, targeting ligands for selectins can be introduced at the external surface of dendrimers, leading to the formation of targeted drug delivery systems. In this regard, galactose surface-coated poly(propylene imine) (PPI) derivatives (PPI differs from DAB only in the ethylenediamine core) were prepared[64] and loaded with the antimalarial drug primaquine phosphate (PP).

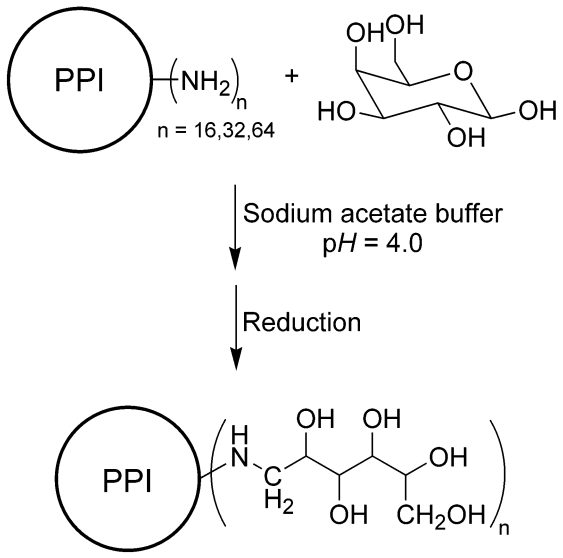
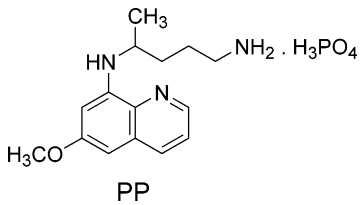


Figure 11. Galactosylation of the poly(propylene imine) dendrimer (PPI) of the third to fifth generation.

Galactose functionalization was carried out by a ring opening reaction followed by a Schiff's base reaction and reduction to secondary amine in sodium acetate buffer as shown in Figure 11.



PP

Galactose has been shown to be a promising ligand for hepatocyte (liver parenchymal cells) targeting because liver cells possess a large number of asialo-glycoprotein (ASGP) receptors that can recognize the galactose units on the oligosaccharide chains of glycoproteins or on chemically galactosylated drug carriers.[65] In this case, polyvalent interaction results in extremely strong binding of ligands to the receptors.

Release rate, hemolytic toxicity, biodistribution, and blood level studies were performed using healthy albino rats of uniform body weight (100 ± 20) with no prior drug treatment. The results indicated that galactose coating of PPI systems increases the drug entrapment efficiency by 5−15 times depending upon the generation of dendrimers. Also, galactose coating prolonged release up to 5−6 days as compared to 1−2 days for uncoated PPI. The hemolytic toxicity, blood level, and hematological studies proved that these carriers are safer and suitable for sustained drug

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delivery. Blood level studies proved the suitability of the carriers in prolonging the circulation and delivery of PP to liver.

The folate receptor is known to be significantly overexpressed over a wide variety of human cancers, and therefore, folate-mediated targeting has been widely applied with a diversity of carriers, including liposomes,[66−68] dendrimers,[69−71] and various polymers and particles[72−79] that are used as drug delivery systems.

Employing the folate receptor for targeting, the folic acid moiety in combination with other functional groups was introduced onto the surface of a third-generation PAMAM dendrimer for the formation of a multifunctional dendrimeric derivatives;[71] in vitro and vivo studies were performed, and the results are summarized below. These derivatives, in addition to the protective PEG chains, also bear the folate moiety at the end of the poly(ethylene glycol) chain which can induce endocytosis in folate receptor-bearing cells.[78−83] Furthermore, in the previously functionalized dendrimer, 12−15 decaborate clusters were covalently attached which can be used for the treatment of cancer in boron neutron capture therapy (BNCT), requiring the selective delivery of ^{10}B to cancerous cells within a tumor. Varying numbers of PEG chains differing in length were linked to these boronated dendrimers to reduce hepatic uptake. Among the prepared combinations, boronated dendrimers with 1−1.5 PEG_{2000} units exhibited the lowest hepatic uptake in C57BL/6 mice [7.2−7.7% injected dose (ID)/g of liver].

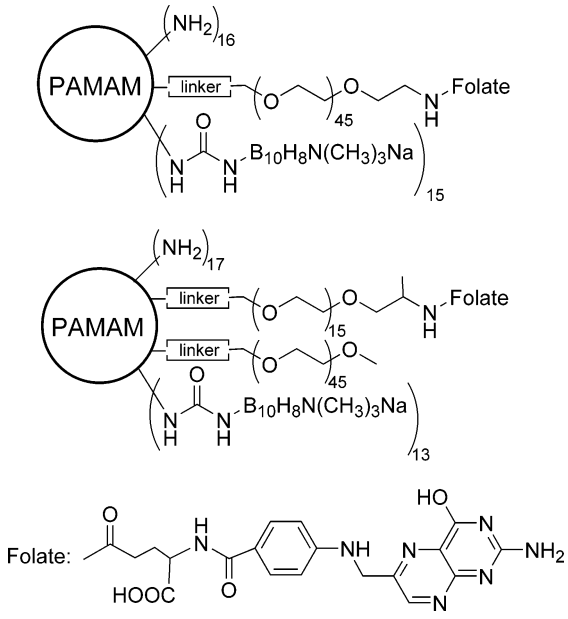

Figure 12. Boronated third-generation PAMAM dendrimer functionalized with the PEG-folate moiety.

Folate receptor-targeted and boronated third-generation poly(amidoamine) dendrimers were prepared as shown in Figure 12. One contains ∼15 decaborate clusters and ∼1 PEG_{2000} unit with a folic acid moiety attached to the distal end, while the other contains ∼13 decaborate clusters, ∼1 PEG_{2000} unit, and ∼1 PEG_{800} unit with folic acid attached to its distal end. In vitro studies using folate receptor (+) KB cells demonstrated receptor-dependent uptake of the latter

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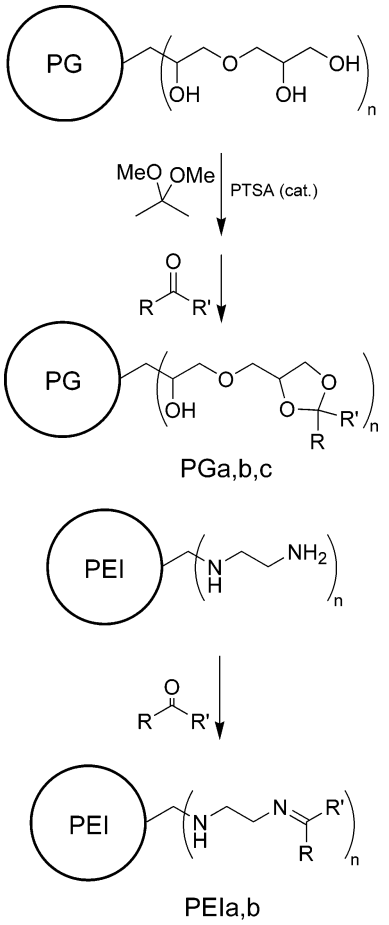


Figure 13. Functionalization of PG and PEI hyperbranched dendritic polymers with various carbonyl compounds listed in Table 4.

folic acid-functionalized derivative. Biodistribution studies with this derivative in C57BL/6 mice bearing folate receptor (+) murine 24JK-FBP sarcomas resulted in selective tumor uptake (6.0% ID/g of tumor) but also high hepatic (38.8% ID/g) and renal (62.8% ID/g) uptake. This indicates that the attachment of a second PEG unit and/or folic acid may adversely affect the pharmacodynamics of this conjugate. It was concluded that the optimal modification of boronated dendrimers and in general of dendrimers bearing PEG chains for reducing RES affinity appears to be a highly complex process that depends on a variety of factors requiring extensive evaluation.

3. From Mono- to Multifunctional Hyperbranched Polymers as Drug Carriers

Employing polyglycerol (PG) and poly(ethylene imine) (PEI) as starting compounds and applying appropriate functionalization, Krämer et al. developed pH-responsive molecular carriers.[84] The concept of pH-responsive carriers may have potential application for selective drug delivery in tissues with a lower pH value (for example, infected or tumor tissue). Polyglycerol and poly(ethylene imine) are randomly branched with a degree of branching of 60−75%,

Table 4. Encapsulation Capacities of Congo Red in Dendritic Nanocarriers Based on PG and PEI[a]

Structure	MWn core [gmol^{-1}]	Shell	Degree of alkylation	Encapsulation capacity
PG	21 000	-	-	-
PGa	21 000	$C_{15}H_{31}$ (O) H	25%	$0.15_{\pm 0.05}$
PGb	21 000	$C_{16}H_{33}$ (O) $C_{16}H_{33}$	45%	$13_{\pm 4}$
PGc	21 000	$C_{16}H_{33}$ (O) $C_{16}H_{33}$	55%	$2_{\pm 0.5}$
PEI	25 000	-	-	$0.02_{\pm 0.005}$
PEIa	25 000	$C_{15}H_{31}$ (O) H	33%	$0.6_{\pm 0.1}$
PEIb	25 000	C_5H_{11} (O) C_5H_{11}	53%	$0.2_{\pm 0.05}$

[a] Taken with permission from ref 84. Copyright 2002 Wiley-VCH.

and they are commercially available. Functionalization of these dendritic polymers was conveniently achieved by a condensation reaction of the 1,2-diol or NH_2 moieties at their external surface with various carbonyl compounds as shown in Figure 13 and Table 4. Several dendritic structures originating from these reactions have been prepared, differing as follows: (a) the type and molecular weight of the core polymer, (b) the structure of the attached peripheral shell, and (c) the degree of alkylation.

The loading capacities, i.e., the number of encapsulated congo red molecules per dendritic nanocarrier, together with their structural features are listed in Table 4. It was found that a minimum core size (ca. 3000 g/mol) and a highly branched architecture are required for successful encapsulation of the guest molecules. For efficient encapsulation, the degree of alkylation should be ∼45−50% and the alkyl chains should have a minimum length (>10 carbons). For example, the conversion of the terminal groups in polyglycerol (PG) with a C16 aldehyde (PGa) containing one alkyl chain per diol unit results in an effective degree of alkyl functionalization of 25% (Table 4) and a poor encapsulation capacity (0.15 congo red molecule). With the same PG core, the ketal-functionalized carrier PGb with two alkyl chains per diol unit and 45% effective alkyl functionalization (Table 4) encapsulates up to 13 congo red molecules. A higher degree of ketal functionalization (55% for PGc, Table 4) indicates an optimal shell density of 45−50%. The exact determination of the encapsulation capacities for the amine-based carriers, PEI (Table 4), was complicated because of the hydrolytic sensitivity of the imine-bound peripheral shell

(84) Krämer, M.; Stumbé, J.-F.; Türk, H.; Krause, S.; Komp, A.; Delineau, L.; Prokhorova, S.; Kautz, H.; Haag, R. pH-responsive molecular nanocarriers based on dendritic core-shell architectures. *Angew. Chem., Int. Ed.* **2002**, *41*, 4252−4256.

in the PEI-based systems, for instance, in PEIb. To avoid hydrolysis, the dye was directly encapsulated from the solid−organic solution interface.

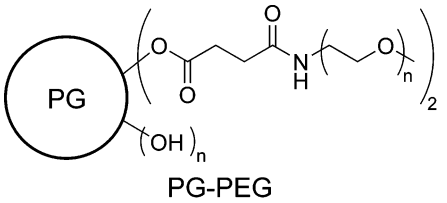
For the complexation of an antitumor drug, mercaptopurine, several oligonucleotides as well as bacteriostatic silver compounds (for example, AgI salts and Ag^0 nanoparticles)[85] have been studied for the potential use of these carriers for drug and gene delivery. Successful encapsulation was observed in all cases by the PEI-based carriers, while complexation was not observed with the PG-based carriers for the same guest molecules.

The objective of developing a pH-sensitive carrier was tested using several buffer solutions for both the acetal- and imine-bound shells. The differences in hydrolyzing the previously prepared dendritic derivatives in certain buffers control the release of their encapsulated compounds. The encapsulated congo red in the carrier PGb was stable for several months at neutral and basic pH values (pH >7). However, an immediate release of the guest molecules occurred in acidic media (pH <3). The imine-based carriers were even more sensitive to an external decrease in pH. In the case of carrier PEIa, the hydrolysis of the shell and the release of the encapsulated guest (namely, congo red) occur over a period of 4 days at pH 6. However, it is stable over several weeks at neutral pH. On the other hand, the hydrolysis of the carrier PEIb and the release of the encapsulated guest occur spontaneously even at pH <7. For PEIb, a slow release is observed after several hours (pH 8, ca. 3 h, 25 °C) or days (pH 12, 2 days, 25 °C) even without acidification. For the PEI-based carrier PEIb, the amount of dye due to imine hydrolysis was followed by IR spectroscopy through the disappearance of the imine signal.

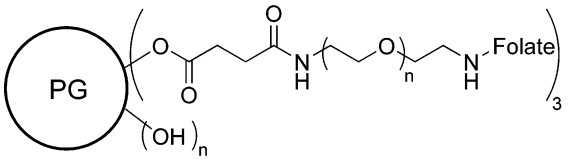
Hyperbranched polyglycerol (PG), exhibiting low toxicity and biocompatibility, was also functionalized, providing a multifunctional drug delivery system. In this nanocarrier, due to its polyether internal structure, release of the encapsulated drug can be salt-triggered. The utmost objective of this hyperbranched polymer functionalization is, as in the case with dendrimers, to simultaneously address the main issues encountered with drugs themselves, as well as with drug carriers, i.e., water solubility, stability in biological milieu, and targeting. For this purpose, PEGylated (PG-PEG) and PEGylated-folate (PG-PEG-folate) functional derivatives of polyglycerol were prepared and investigated as prospective drug delivery systems.[86]

To investigate this possibility, in addition to the use of the sensitive pyrene probe, tamoxifen (TAM), an anticancer hydrophobic drug, was employed.

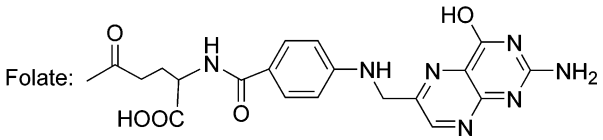
Tamoxifen is a nonsteroidal anti-estrogen drug, which is

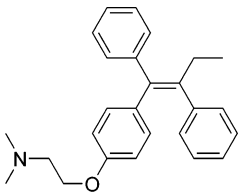


PG-PEG



PG-PEG-Folate





TAM

widely used in the treatment and prevention of breast cancer.[87,88] Its encapsulation and release were comparatively investigated for the parent polyglycerol (PG), PG-PEG, and the multifunctional PG-PEG-folate derivative. The solubility of TAM in water was found to be 1.9×10^{-6} M. Its solubility increases by a factor of 5 when it is solubilized in a PG solution (Table 5). The solubility of TAM is considerably further enhanced by a factor of 65 in the presence of PG-PEG. This significant solubility increase indicates that TAM is solubilized not only inside the hyperbranched interior but also inside the covalently bound poly(ethylene glycol) chains. This behavior is analogous with previous results with PEGylated dendrimeric derivatives[52,55] encapsulating the hydrophobic anti-inflammatory corticosteroid drugs. This further establishes the fact that the introduction of the poly(ethylene glycol) chains in general enhances the solubilization efficiency of dendritic polymers. It is worth mentioning that for PG-PEG-folate an ∼1300-fold increase in TAM solubility was observed.

The release of TAM from PG and its derivatives was induced by titrating the aqueous PEGylated hyperbranched polymers with aqueous NaCl solutions in analogy with the experiments with PEGylated dendrimers.[55] In principle, solubilized molecules can be replaced with metal ions, and it is, therefore, necessary to investigate whether sodium

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Table 5. Solubility of Tamoxifen in PG, PG-PEG, and PG-PEG-Folate Aqueous Solutions[a]

hyperbranched polymer	[polymer] (M)	[tamoxifen] (M)
PG	1.0×10^{-3}	9.6×10^{-6}
PG-PEG	1.0×10^{-3}	1.23×10^{-4}
PG-PEG-folate	1.0×10^{-3}	2.48×10^{-3}

[a] Taken with permission from ref 86. Copyright 2006 Wiley-VCH.

cation complexation can probably cause premature release of the drug in the extracellular fluids before the nanocarrier, loaded with the drug, reaches the target cell. By this titration, TAM was released and suspended in the bulk aqueous phase. In the addition of a 0.142 M NaCl solution, 39 and 24% of the solubilized TAM in PG and PG-PEG, respectively (Figure 14), was released in the aqueous media. Under the same conditions and in the presence of PG-PEG-folate, only 6% of the solubilized TAM was released (Figure 14). It should, therefore, be noted that for the most elaborated multifunctional derivative prepared in this study, i.e., PG-PEG-folate, most of TAM remained encapsulated in the polymer, and it is not released in the extracellular fluid at a NaCl concentration of 0.142 M. Therefore, this nanocarrier can reach target cells appreciably loaded with TAM.

4. From Mono- to Multifunctional Dendritic Polymers as Gene Carriers

In recent years, numerous gene delivery systems based on viral[89−92] and nonviral[92−97] vectors have been developed and tested. However, safety issues concerning viral vectors have led to a careful reconsideration of their application for human clinical trials and prompted the use of synthetic systems. Moreover, viral vectors experience significant limitation in large-scale production and the available size of DNA they can carry. To address these problems, nonviral gene delivery systems such as cationic polymers or cationic lipids, liposomes, or cationic dendrimers have attracted significant interest for achieving a breakthrough in the development of an effective gene carrier. It has to be noted that synthetic nonviral carriers of genetic material present insignificant risks of genetic recombinations in the genome. Transfection with tailor-made synthetic vectors may exhibit low cell toxicity, high reproducibility, and ease of application.

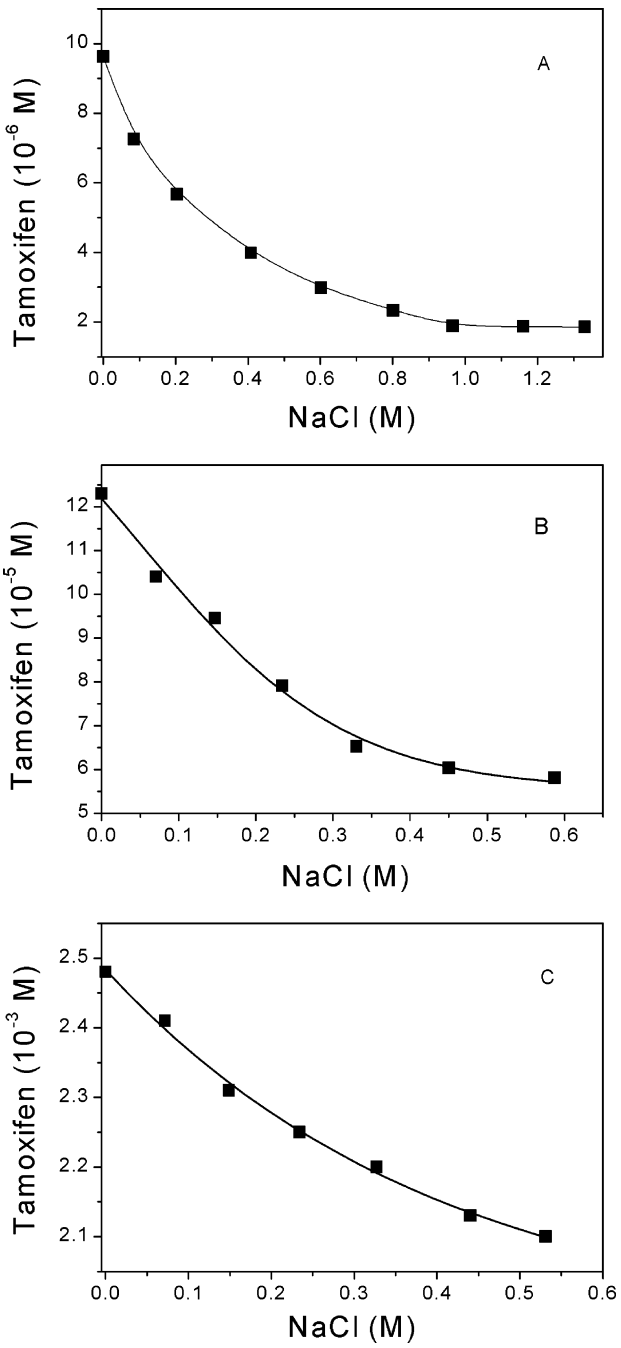

Figure 14. Tamoxifen concentration in PG (A), PG-PEG (B), and PG-PEG-folate (C) aqueous solutions (1×10^{-3} M) as a function of the amount of added NaCl solution. Reprinted with permission from ref 86. Copyright 2006 Wiley-VCH.

(89) Verma, I. M.; Somia, N. Gene therapy: Promises, problems and prospects. *Nature* **1997**, *389*, 239−242.
(90) Lotze, M. T.; Kost, T. A. Viruses as gene delivery vectors: Application to gene function, target validation, and assay development. *Cancer Gene Ther.* **2002**, *9*, 692−699.
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However, the currently developed synthetic vectors exhibit disadvantages, due to their generally low effectiveness compared to viral vectors and to their inability to target gene expression. For effective gene expression, genes must be transferred in the interior of the nucleus of the cell and this procedure has to circumvent a series of endo- and exo-cell hurdles. Among these obstacles are cell targeting, effective transport of the carriers together with attached genetic material through cell membranes, and the need to release carriers from the endosome following endocytosis. Synthetic carriers address to some extent these difficulties without, however, completely achieving the optimal objective.

The delivery of genetic material to cells was achieved by following a strategy analogous to that employed for conventional drugs. Specifically, the method involves molecular engineering of a dendritic surface and/or the core with the objective of obtaining functional dendritic polymers, which should be positively charged, relatively stable in a biological environment, and nontoxic, exhibiting targeting properties and having the ability to be effectively transported through cell membranes. Furthermore, the dendritic polymer−DNA complex should be released from the endosome following endocytosis. Before discussing specific examples, we should mention that compared to the relatively unstable liposomes, the dendritic polymers are stable nanoparticles. In addition, dendrimers, due to their dependence of size on generation, provide a tool for investigating and affecting transfection efficiency.

As recently reviewed,[98] the use of even unmodified amino-terminated PAMAM or DAB dendrimers as gene transfer agents enhances the transfection of DNA into the cell nucleus. The exact structure of these host−guest binding motifs has not been determined in detail, but it is presumably based on acid−base interactions between the anionic phosphate moieties on the DNA backbone and the primary and tertiary amine moieties of the dendrimers, which are positively charged under physiological conditions. It is interesting to note[99] that partially fragmented dendrimers are more appropriate for gene delivery than the intact dendrimers, and a fragmentation (or activation step) consisting of hydrolytic cleavage of the amide bonds is needed to enhance the transfection. It has been concluded from several investigations that the spherical shape of dendrimers is not advantageous in gene delivery. This agrees with earlier work in which "fragmented" PAMAM dendrimers exhibit superior transfection efficacy in comparison with the spherical "intact" dendrimers.[98] This property can open the application for the less expensive hyperbranched polymers as gene delivery systems. Partially degraded dendrimers have a more flexible structure and form a more compact complex with DNA, which is preferable for gene delivery by the endocytotic pathway.[100] In addition, it is generally found that the maximum transfection efficiency is obtained with an excess of primary amines to DNA phosphates, yielding a positive net charge of the complexes.[98] The more flexible higher-generation DAB dendrimers are found to be too cytotoxic for use as nonviral gene vectors; however, the lower-generation dendrimers are well-suited for gene delivery due to their effective transfection[101] (Figure 15).

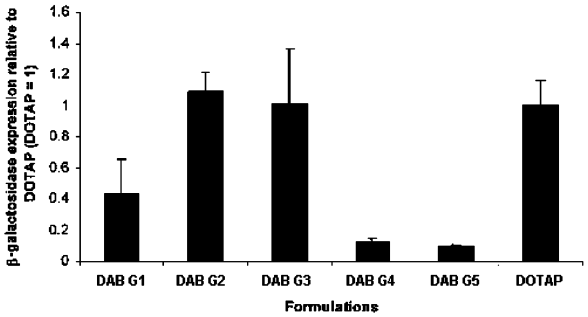

Figure 15. Transfection efficacy of poly(propylene imine) dendrimers of various generations (G1−G5) relative to *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammoniummethyl sulfate (DOTAP) in the A431 cell line. Reprinted with permission from ref 101. Copyright 2002 American Association of Pharmaceutical Scientists.

Following these introductory remarks, molecularly engineered PAMAM-OH dendrimers have been prepared. These are structurally similar to PAMAM, except that their surface amino groups have been replaced with hydroxyl groups.[102] The absence of surface primary amino groups in PAMAM-OH renders this polymer nearly neutral which might be advantageous in terms of cytotoxicity. However, PAMAM-OH is nearly unable to form complexes with DNA (polyplexes) because of the low pK_a of interior tertiary amino groups.[103] For this purpose, the synthesis and characterization of internally quaternized PAMAM-OH (QPAMAM-OH) with various quaternization percentages of the tertiary amino groups have been reported.

The internal quaternary ammonium groups of QPAMAM-OH will interact with negatively charged DNA, while preserving a neutral polymer and/or a polyplex surface. These QPAMAM-OH/DNA polyplexes were round-shaped with the

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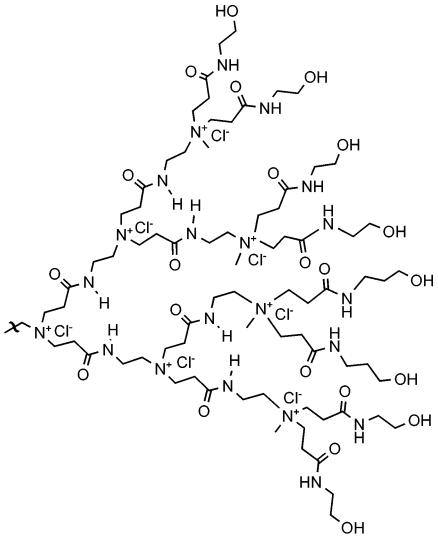

QPAMAM-OH

more compact and small particles formed as the charge ratio increased. Although the transfection efficiency of functional QPAMAM-OH derivatives was lower by 1 order of magnitude than that of the parent PAMAM (Figure 16), the QPAMAM-OH/DNA particles exhibited reduced cytoxicity compared with PAMAM and PEI. This shielding of the interior positive charges by surface hydroxyls may be the reason for this behavior.

The major problem for the nonviral gene delivery systems is their lower efficiency compared to viral vectors. Many methods have been used to overcome such an obstacle, including linking cell-targeting ligands or cell-penetrating peptides as efficient vectors for intracellular delivery of bioactive molecules.[104] Arginine-rich peptides have exhibited an enhanced translocation ability, which was attributed to the presence of the guanidinium moiety,[57,104−108] a structural feature of L-arginine, which is capable of forming hydrogen bonds coupled with electrostatic interactions[109] with phosphate or carboxylic groups located at the surface of cell membranes.

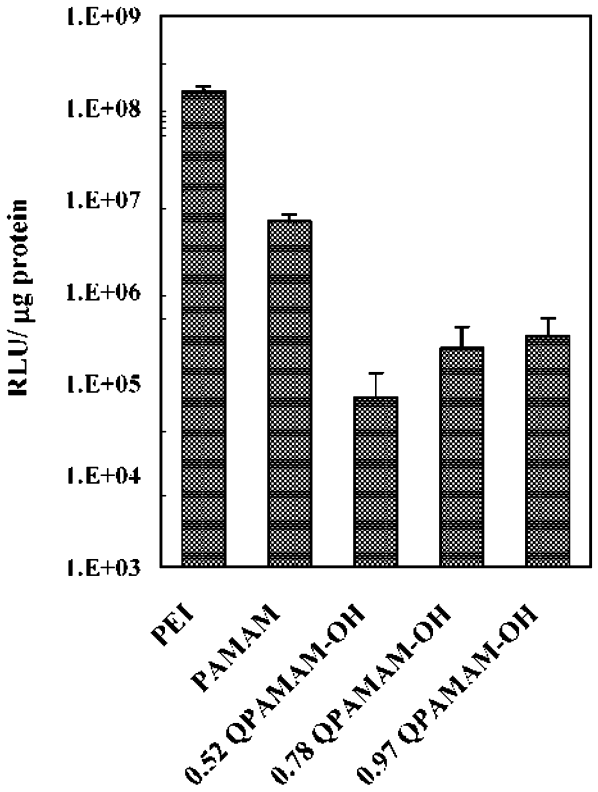

Figure 16. Transfection efficiency of PEI, PAMAM, and QPAMAM-OH dendrimers with degrees of quaternization of 52, 78, and 97% in 293T cells at a charge ratio (±) of 6. Data are expressed in relative light units (RLU) per microgram of protein. Reprinted from ref 102. Copyright 2003 American Chemical Society.

The synthesis of the L-arginine-grafted PAMAM dendrimer (PAMAM-Arg) has been reported recently,[110] which consisted of a PAMAM scaffold the surface of which was covered with L-arginine residues (Figure 17). Via the introduction of arginine moieties onto the PAMAM surface, gene delivery efficiency is greatly enhanced in comparison to that of starting PAMAM (Figure 18). It was comparable to that of PEI for HepG2 and primary rat vascular smooth muscle cells and was more efficient in the case of Neuro 2A cells than PEI and Lipofectamine. L-Lysine-grafted PAMAM (PAMAM-Lys), which was used as a control, exhibited a slightly better transfection efficiency in HepG2 cells than PAMAM, while increased efficiency was not observed in primary cells. In conclusion, a polyvalent arginine-functionalized PAMAM is easily prepared and possesses outstanding transfection efficiency with relatively

(104) Futaki, S. Membrane-permeable arginine-rich peptides and the translocation mechanisms. *Adv. Drug Delivery Rev.* **2005**, *57*, 547−558.

(105) Vivès, E.; Brodin, P.; Lebleu, B. A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. *J. Biol. Chem.* **1997**, *272*, 16010−16017.

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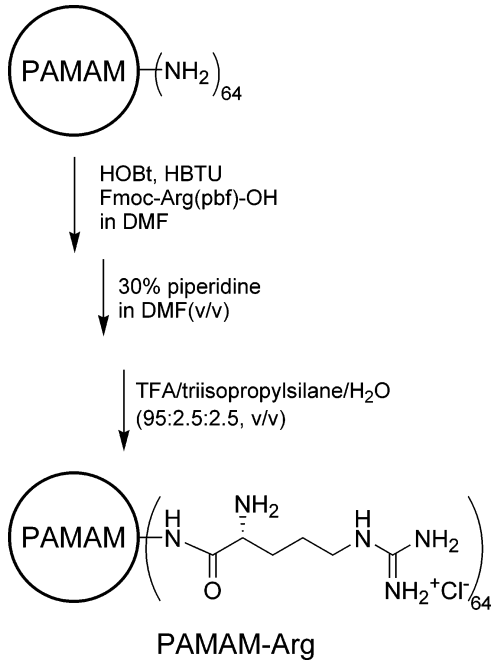

Figure 17. Synthetic pathway for the introduction of L-arginine at the external surface of the fourth-generation PAMAM dendrimer.

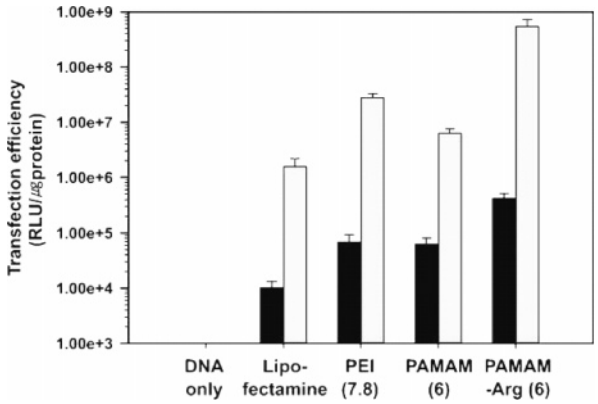

Figure 18. Transfection efficiency for Neuro 2A cell lines (1×10^5 cells/well). The amount of DNA per well was 0.2 (black) and 1.0 μg (white). Values in parentheses represent the charge ratio (N:P) of dendrimer/plasmid DNA complexes. The luciferase expression mediated by reagents was assessed under each optimal condition. Results are expressed as mean $\pm$ SD ($n = 3$). Reprinted with permission from ref 110. Copyright 2004 Elsevier B.V.

low cytotoxicity. These properties would make PAMAM-Arg a promising nonviral vector for both in vitro and in vivo use. PAMAM-Arg could potentially be used as a dendritic nanocarrier encapsulating or incorporating small molecules, peptides, proteins, oligonucleotides, and plasmids that are deficient in cell penetration.

In a recent study,[111] a fourth-generation poly(propylene imine) dendrimer has been completely or partially functionalized with guanidinium groups. For the partially guanidinylated derivatives, the toxic primary amino groups of the dendrimers were reacted with propylene oxide, affording the corresponding hydroxylated derivatives. Five derivatives bearing 0, 6, 12, 24, or 32 guanidinium groups have been prepared. These guanidinylated dendrimers interacted with plasmid DNA, affording the corresponding dendriplexes. The transfection efficiency was assessed by employing HEK 293 and COS-7 cell lines, while the serum effect was studied in HEK 293 cells. It was found that complete replacement of primary amino groups with the hydroxylated moieties resulted in a complete loss of transfection efficiency. On the other hand, guanidinylation of the parent dendrimer resulted in the significant enhancement of its transfection efficiency, this enhancement being dependent on the number of guanidinium groups per dendrimer, the cell line used, and the presence or absence of FBS. The fully guanidinylated dendrimer exhibited the best transfection efficiency under all the conditions that were studied. This efficiency has been attributed to the enhanced penetrating ability of the guanidinylated dendrimers due to the accumulation of the guanidinium group at the dendrimeric surface. It was also found that the derivative with 12 guanidinium groups exhibited the lowest toxicity. The reduction in toxicity was apparently attributed to the decrease in the number of external primary amino groups coupled with the presence of hydroxylated moieties located at the dendrimeric surface. The functionalization strategy that was employed leads to dendrimeric derivatives that combine satisfactory transfection efficiency with minor cytotoxicity.

In this regard, to enhance the transfection efficiency of PAMAM dendrimers, phenylalanine or leucine moieties have been introduced at their chain ends.[112] Efficient transfection of cells was achieved through synergy of the proton sponge effect, which is induced by the internal tertiary amines of the dendrimer, and the hydrophobic interaction by the hydrophobic amino acid residues in the dendrimer surface. Specifically, dendrimers bearing 16, 29, 46, and 64 terminal phenylalanine residues were prepared by the interaction of fourth-generation PAMAM with L-phenylalanine. The transfection activity of these phenylalanine-modified dendrimers, (Phe)64-G4, for CV1 cells, an African green monkey kidney cell line, increased in a manner concomitant with the increasing number of the terminal phenylalanine residues, except for the dendrimer with 64 phenylalanine residues, which exhibited poor water solubility and hardly formed a complex with DNA at neutral pH. However, under weakly acidic conditions, the dendrimer with 64 phenylalanine residues formed a complex with DNA, thereby achieving highly efficient transfection. In contrast, the attachment of L-leucine residues, (Leu)63-G4, did not improve the transfection efficiency compared to that of the parent dendrimer

(111) Tziveleka, A.; Psarra, A.-M. G.; Tsiourvas, D.; Paleos, C. M. Synthesis and Characterization of Guanidinylated Poly(propylene imine) Dendrimers as Gene Transfection Agents. *J. Controlled Release*, in press.

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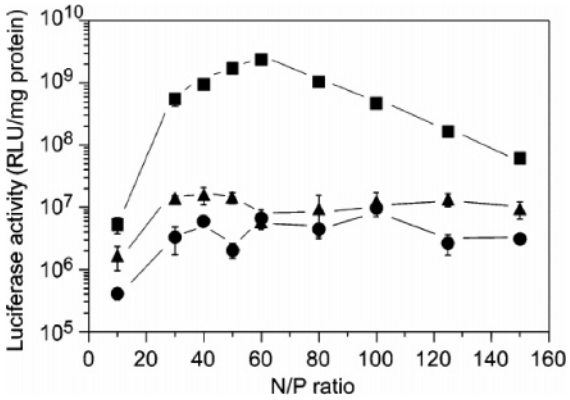


Figure 19. Comparison of the transfection activity of leucine- and phenylalanine-modified PAMAM dendrimers. Luciferase activities of CV_1 cells treated with (Phe)64-G4 polyplex prepared at pH 5.0 (■) and (Leu)63-G4 polyplexes prepared at pH 7.4 (▲) and 5.0 (●) containing 1 μg of DNA with varying N:P ratios are shown. Each bar represents the mean ± SD (n = 3). Reprinted from ref 112. Copyright 2005 American Chemical Society.

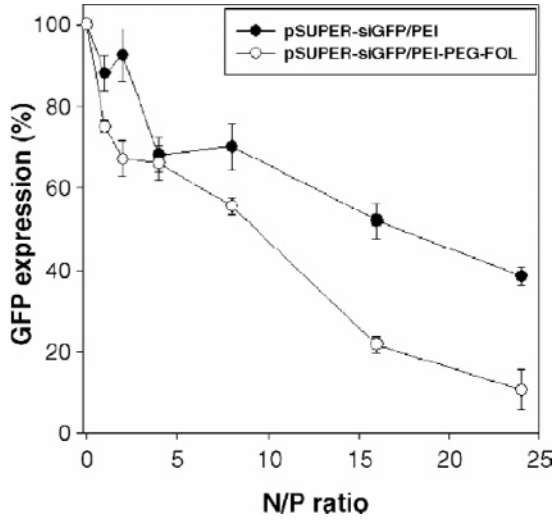
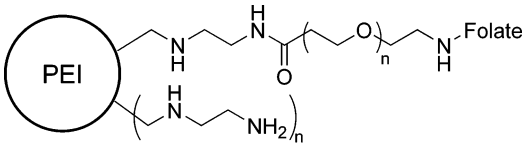


Figure 20. GFP gene inhibition efficiency of pSUPER-siGFP/PEI and pSUPER-siGFP/PEI-PEG-folate complexes as a function of N:P ratio against GFP-KB cells. Reprinted with permission from ref 113. Copyright 2005 Elsevier B.V.

(Figure 19). This is probably attributed to the relatively lower hydrophobicity of this amino acid. The phenylalanine-modified dendrimer exhibited a higher transfection activity and a lower cytotoxicity than some widely used transfection reagents. For this reason, the phenylalanine-modified dendrimers are considered to be promising gene carriers.

The structural features required for an effective dendritic gene vector are possibly satisfied in a PEI-poly(ethylene glycol)-folate derivative (PEI-PEG-folate), which was recently synthesized,[113] and its efficiency as a gene carrier was tested.



This multifunctional hyperbranched PEI (MW = 25 000) could simultaneously combine protective and targeting properties. In this study, the PEI-PEG-folate nanocarrier was tested for its capacity to complex with plasmid DNA and be transfected to folate receptor-overexpressing cells (GFP-KB cells) that produce exogenous green fluorescent protein (GFP). A special plasmid system (pSUPER-siGFP) that carried a siRNA-expressing sequence, used for inhibiting the expression of exogenous GFP in mammalian cells, was prepared. The pSUPER-siGFP/PEI-PEG-folate complexes inhibited GFP expression of KB cells more effectively than pSUPER-siGFP/PEI complexes (Figure 20). These results indicated that folate receptor-mediated endocytotosis is a major pathway in the process of cellular uptake.

5. Biodegradable Dendritic Polymers with Prospective Molecular Engineering Properties

In the previous sections of this review, we have discussed the modification of four basic dendritic polymers, PAMAM, DAB, PG, and PEI, and to what extent these modifications affect their properties as drug and gene delivery systems. It has been established that the systems that have been developed are rather successful since they have higher water solubility, exhibit better stability in biological environments, are less toxic compared to starting dendritic polymers, are nonimmunogenic, and exhibit targeting ability for specific cells. The systems, however, are not biodegradable which is undesirable primarily for long-term applications. For this reason, attempts to prepare biodegradable dendritic polymers under facile experimental conditions have been made.

In this regard, Fréchet et al.[114,115] have prepared three polyester dendritic systems based on 2,2-bis(hydroxymethyl)-propanoic acid. These three polymers are highly water-soluble and nontoxic and therefore promising as prospective drug delivery systems. The first polyester dendrimer, PE, bears 32 free hydroxyl groups and has a molecular weight of 3700, while the second, PE-EO, has a molecular weight of 12182. The second dendritic system was prepared from PE by introducing tri(ethylene glycol) moieties at its external surface. Furthermore, although these dendritic polymers are biodegradable, their sterically hindered ester bond makes

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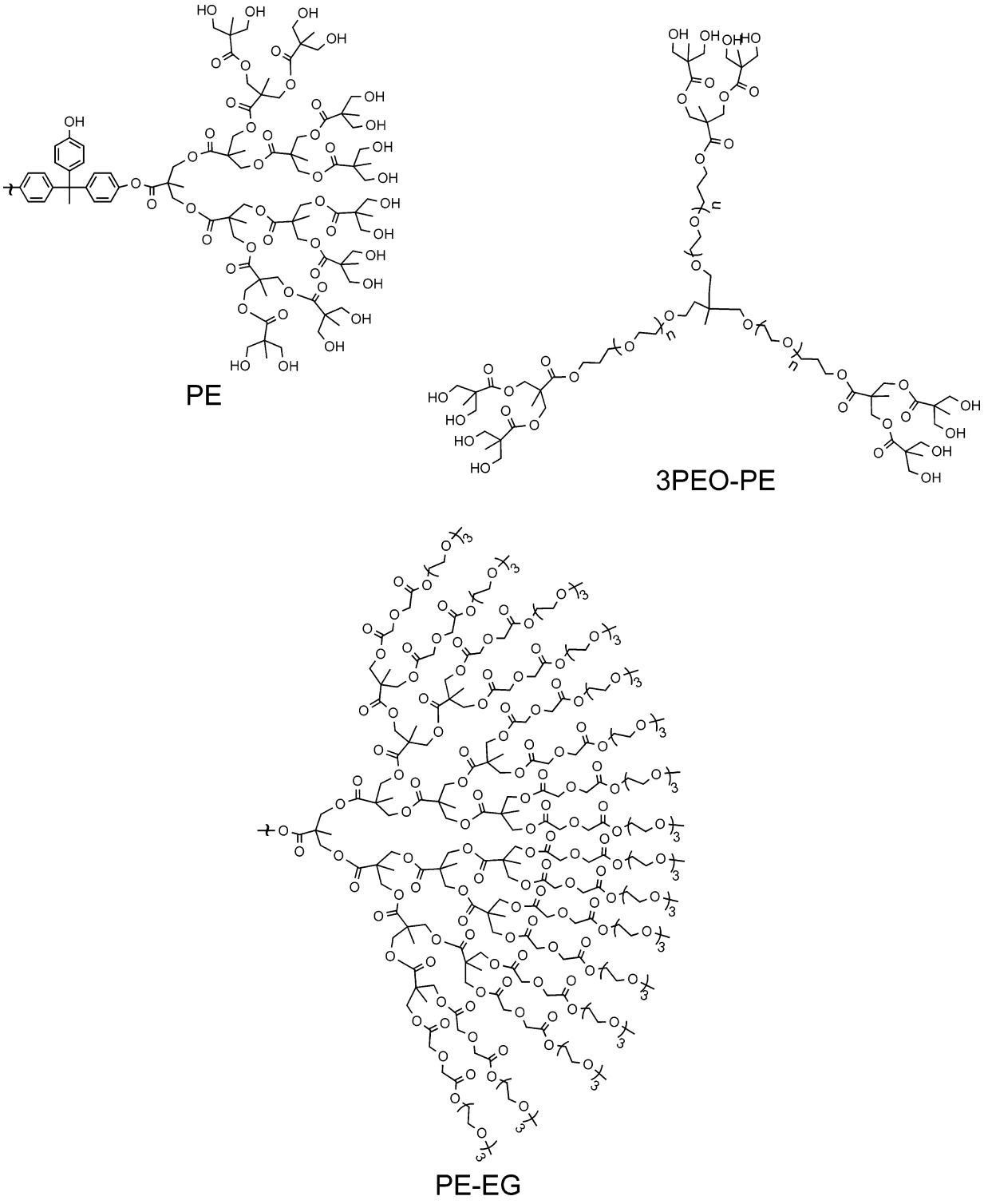
Chart 2



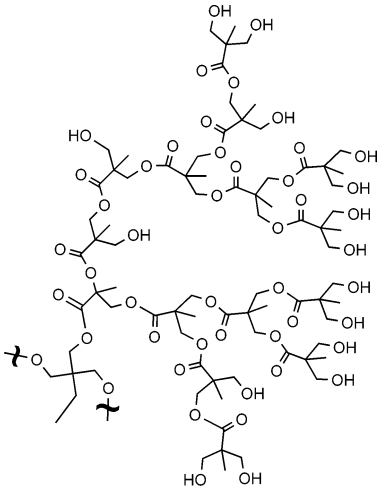
their backbone relatively stable toward both nucleophilic attack and acid-catalyzed hydrolysis. In this manner, prolonged biodegradability is obtained which may be beneficial for the controlled release behavior of the system. The third polyester derivative, 3PEO-PE, has a molecular weight of 23500 and is composed of a three-arm poly(ethylene oxide) star and polyester dendrons. This derivative exhibited the longest circulatory half-life (72 min) compared to the first two polyester derivatives and was selected for further investigation (Chart 2).

To this last polyester was covalently attached the anticancer drug doxorubicin (DOX) via an acid-labile hydrazone linkage. The cytotoxicity of the drug was significantly reduced by 80−98% when it was attached to the polyester. Doxorubicin, however, can rapidly be released from its carrier at the pH of the lysosome. Uptake of the doxorubi-

cin−polyester conjugate by cells was observed by fluorescence confocal microscopy. In biodistribution experiments with this doxorubicin conjugate, accumulation in any vital organ was not observed, including the liver, heart, and lungs. This is a significant improvement over the administration of the free drug, which partitions into organs such as liver and heart. However, a higher-molecular weight system must be prepared to further increase the circulation half-life for effectively exploiting the enhanced permeability and retention (EPR) effect.

Analogous dendritic hyperbranched polyesters were recently prepared,[116] and their synthesis was based again on 2,2-bis(hydroxymethyl)propanoic acid as an AB_2 monomer and 2,2-bis(hydroxymethyl)-1,3-propanediol as a central core. They are supplied with various molecular weights, having a different number of primary hydroxyl groups, i.e., 16, 32, and 64 for Boltorn H20, H30, and H40, respectively, which are susceptible to functionalization.

Two glycodendritic structures[117] have been prepared with 16 and 32 mannose moieties originating from Boltorn H20 and H30 hyperbranched polymers which were used as controls. These glycodendrimers are water-soluble, exhibit low toxicity, and have the ability to interact with lectin receptors and therefore can be considered promising candidates for drug delivery. In addition, preliminary experiments have shown that the water-insoluble Boltorn H40 is becoming water-soluble through PEGylation and is encapsulating



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hydrophobic drugs (our unpublished results). It is therefore anticipated that further studies with such biodegradable polymers will soon appear in the literature.

6. Concluding Remarks

Molecular engineering of basic dendrimeric and hyperbranched polymer scaffolds resulted in the preparation of nanocarriers of low toxicity, significant encapsulating capacity, a specificity for certain biological cells, and the ability to be transported through their membranes. Depending on the degree and type of functionalization, products that fulfill one or more of the above characteristics were prepared. Polyvalent interactions attributed to the presence of the functional groups, in close proximity, on the external surface of the dendritic polymers are crucial in the induction of these favorable transfection properties.

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