4. Dendritic core-multishell architectures with PG-amine core

4.1. Introduction

Core-multishell architectures with PG-amine as a core were synthesized for two reasons. First, the presence of hyperbranched poly(ethylene imine) in the structure of the nanotransporters raised the question of the biocompatibility of the polymers. PEIs have been widely used in the environment since more than 40 years and no sign of acute or chronic toxicity has been reported. The LD_{50} was established on the level of 4 g / kg by oral application in rats and rabbits.[400] Nevertheless, the cytotoxicity data showed PEI toxicity on the cellular level. This toxicity might be a consequence of the endo/lysosomal enzyme release into the cytoplasm, consecutive to feeding a cell with membrane-disrupting particles.[400,401] This should be considered as an important drawback for the use of PEI for *in vivo* applications. The PEI toxicity can be reduced up to 100 times by functionalization with poly(ethylene glycol) chains.[402-405] Nevertheless, the possibility to avoid the use of PEI and exchange it with biocompatible hyperbranched polyglycerol should result in a fully biocompatible material.

The second reason to use PG cores was to understand the influence of the core type on the transport capacities of the polymers. In the past, both types of hyperbranched polymers (PG and PEI) were used in core-shell systems and both proved good encapsulations abilities.[4,251,354,356,357,359,361] Thus, the universal abilities of the new type of multishell systems were assumed to remain unchanged, although it was still questionable if the aggregates obtained ealier for PEI systems aggregates still played the major role in host-guest interactions. An answer to this question would allow a better understanding of the properties and encapsulation mechanism of liposome-like polymers with polar-nonpolar-polar polarity gradient. Especially in the case of polar ionic guest molecules such as congo red (anionic) the influence of the cationic core should be explained for a better localization of the encapsulation site inside the polymer, which will be discussed in chapter 6.

4.2. Synthesis of the core-multishell architectures with PG core

The hyperbranched polyglycerol (PG) used in this work was obtained by anionic ring opening polymerization of glycidol with 1,1,1-Tris(hydroxymethyl)propane used as a starter. The molecular weight of the PG core was 10000 g mol^{-1} (PG_{10000}) with a degree of branching (*DB*) of 1.7. A single polymer contains approximately 128 -OH groups. The PG_{10000} core was chosen as an equivalent for PEI_{10500} to allow a better comparison of the two systems. Additionally, higher TC ratios obtained for nanotransporters with bigger PEI core permit one

to assume that polymers with PG_{10000} core would also show better TC values than polymers with a smaller PG.

In order to obtain core-multishell architectures with PG core two different synthetic approaches were tested.

In the first method the direct coupling of the mPEG-C_{18} amphiphiles to the polyglycerol hydroxyl groups was tried. The esterification was performed in a melt reaction at 170 °C under the vacuum (5×10^{-2} mbar). The reaction was initially tested with PG_{10000} and stearic acid (octadecanoic acid). As a result, a PG-C_{18} core-shell system was obtained. Despite of this, the expected core-multishell product was not formed from pure PG and $C_{18}mPEG_6$. The reaction was performed for 3 h and led to the nanotransporters with a very low DF_{PG} of 0.10 – 0.15. Prolongation of the reaction time resulted in material that was partially or not soluble. This might be due to a crosslinking reaction between PG cores *via* 1,18-octadecanioc diacid bridges, which might result from the decomposition of amphipiles or transesterification. Reaction under Dean-Stark conditions in toluene did not result in the core-shell architecture, which was probably due to insolubility of the PG in organic solvent.

To avoid the synthetic problems with the esterification reaction an alternative route of core-multishell nanotransporters was developed. In this approach a PG modified to PG-amine was used as a core. This allows the same coupling method of amphiphiles as used for PEI polymers (Scheme 15). The polyglycerylamine ($PG_{10000}NH_2$) was obtained in a 3 step procedure from PG_{10000}. Most of the published works on the transformation of 1,2-diols into the corresponding amine groups used the route of alcohol activation, aziridation, and Staudinger reaction.[406-408] Within this work the activation step was performed *via* mesylation of polyglycerol in pyridine a with following dialysis in acetone. *O*-Mesylpolyglycerol was obtained with quantitative conversion and good yields. The mesylate is a good leaving group which allows nucleophilic substitution with sodium azide in DMF at 60 °C. To obtain the full conversion of -OMs into azide groups a four-time excess of sodium azide was used. The excess of azide was easily removed by filtration and dialysis in $CHCl_3$. Polyglycerylamine was obtained by a Staudinger reduction by addition of 1.5 eq of triphenylphosphine (PPh_3) into the solution of PG-azide in THF / water mixture (the formation of N_2 was observed). The product was purified by dialysis.

The loading of PG with amino groups can be controlled by varying the amount of mesylchloride (MsCl) used in the first step. In contrast to PEI core polymers the degree of functionalization for PG-core polymers was calculated only as DF_A. No DF calculations were performed, although the PG structure let one distinguish between primary and secondary -OH groups. The degree of functionalization of PG core with amine groups is marked as a DF_{NH2} – degree of amination (Equation 8, where $n_{\text{-OMs}}$ is the average number of mesylate groups per PG molecule) for an easier differentiation from DF and DF_A. Degree of

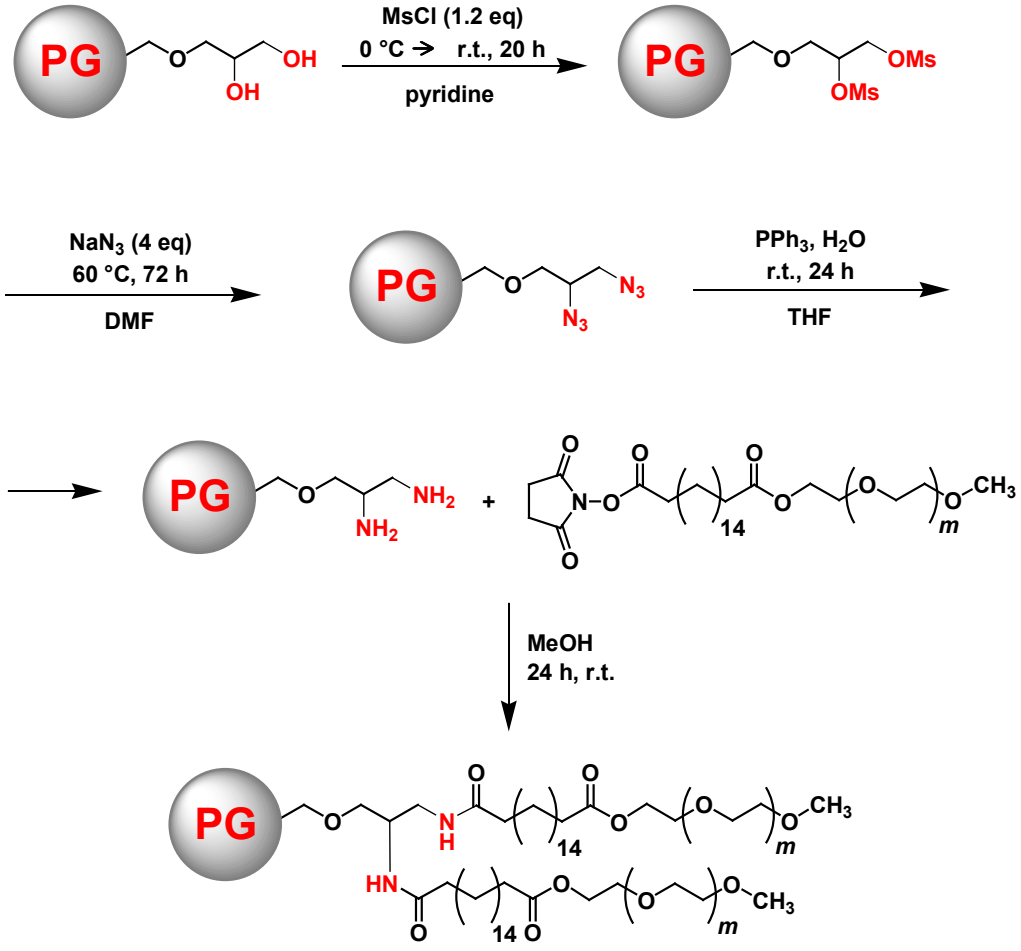
functionalization of PG-amine with amphiphiles was calculated as the percentage of amine group of PG-NH_2 converted into the amide bonds and was marked as DF_{PG} (Equation 9, where $n_{\text{-NHCO-}}$ is the average number of amine group converted into the amide bonds which is equal to the number of amphiphilic chains covalently bounded to the core). Polymer names were coded as $PG_x(\text{-}NH_2)_a(C_{18}mPEG_y)_b$ where x is the M_n of PG core, y is the number of glycol units in mPEG chain, a is a value of DF_{NH2}, and b is a value of DF_{PG}.

$$DF_{NH2} = \frac{n_{-OMs}}{128} \qquad \text{(Equation 8)}$$

$$DF_{PG} = \frac{n_{-.NHCO-}}{128 \times DF_{NH2}} \qquad \text{(Equation 9)}$$

The average number of -OMs groups per polymer was calculated from the ^{1}H NMR spectra. This calculation is based on the comparison of the integration of peaks in the region 3.3 – 5.1 ppm (PG-backone) and 3.0 – 3.15 ppm (-OMs). The integration value of PG signal is divided by 625 (average number of protons per single PG_{10000}) which gives an average intensity per one PG proton. In the next step the integration of the -OMs signal is divided by the intensity of a single proton of PG multiplied by 3. As result the average number of mesylate groups per polymer is obtained ($n_{\text{-OMs}}$). Due to the quantitative conversion (confirmed by 1H NMR) of -OMs to azide and azide to amine it was assumed that $n_{\text{-OMs}}$ is equal to the average number of amine groups per PG.

Three PG-amines with different degrees of amination were synthesized by the described reaction pathway: $PG_{10000}(\text{-}NH_2)_{0.5}$ (with ~64 amine groups), $PG_{10000}(\text{-}NH_2)_{0.7}$ (with ~90 amine groups), and $PG_{10000}(\text{-}NH_2)_{0.9}$ (with ~115 amine groups).



Scheme 15. Four step synthesis of core-multishell architectures with PG-amine core: $PG_{10000}(\text{-}NH_2)_{DFNH2}(C_{18}mPEG_{m+1})_{DFPG}$; $m \approx 5, 13$; $DF_{NH2} = 0.50 – 0.90$ and $DF_{PG} = 0.70 – 1.00$. In the first step -OH groups are converted into mesylate with mesylchloride in pyridine. Then azide groups are synthesized by a reaction with sodium azide in DMF at 60 °C for 72 h. Polyglycerylamine is obtained in the third step by the Staudinger reaction with PPh_3 in THF with 15 % of H_2O addition. In the final step the activated (HONSu) amphiphilic molecules are coupled to the PG-NH_2 in MeOH at r.t.

Coupling between the PG-amine core and the activated amphiphile ((ONSu)$C_{18}mPEG_x$) was performed in methanol at r.t. for 24 h. The reaction conditions were identical like those in the synthesis of core-multishell architectures with the PEI cores. For the reaction the same $mPEG_xC_{18}ONSu$ amphiphiles synthesized as described in chapter 3.2. were used. The final product was purified by dialysis in MeOH for 24 h.

The determination of the degree of functionalization was difficult for polymers with PG-NH_2 core. Both PG-amine and mPEG exhibit ^1H NMR signals in the same region. Therefore the calculation of the DF_{PG} from ^1H NMR is combined with a large error (Figure 40). Thus DF_{PG} was calculated for all synthesized polymers from the stoichiometrical amounts of PG-NH_2 and mPEG-C_{18}-ONSu applied. These values have also been used to calculate the respective molecular weights of each polymer. In total, 8 different polymers

were synthesized with 3 different DF_{NH2} cores and 2 different mPEG lengths ($mPEG_6$ and $mPEG_{14}$) (Table 10). All polymers were very easily soluble in various types of solvent identical to the polymers based on PEI core.

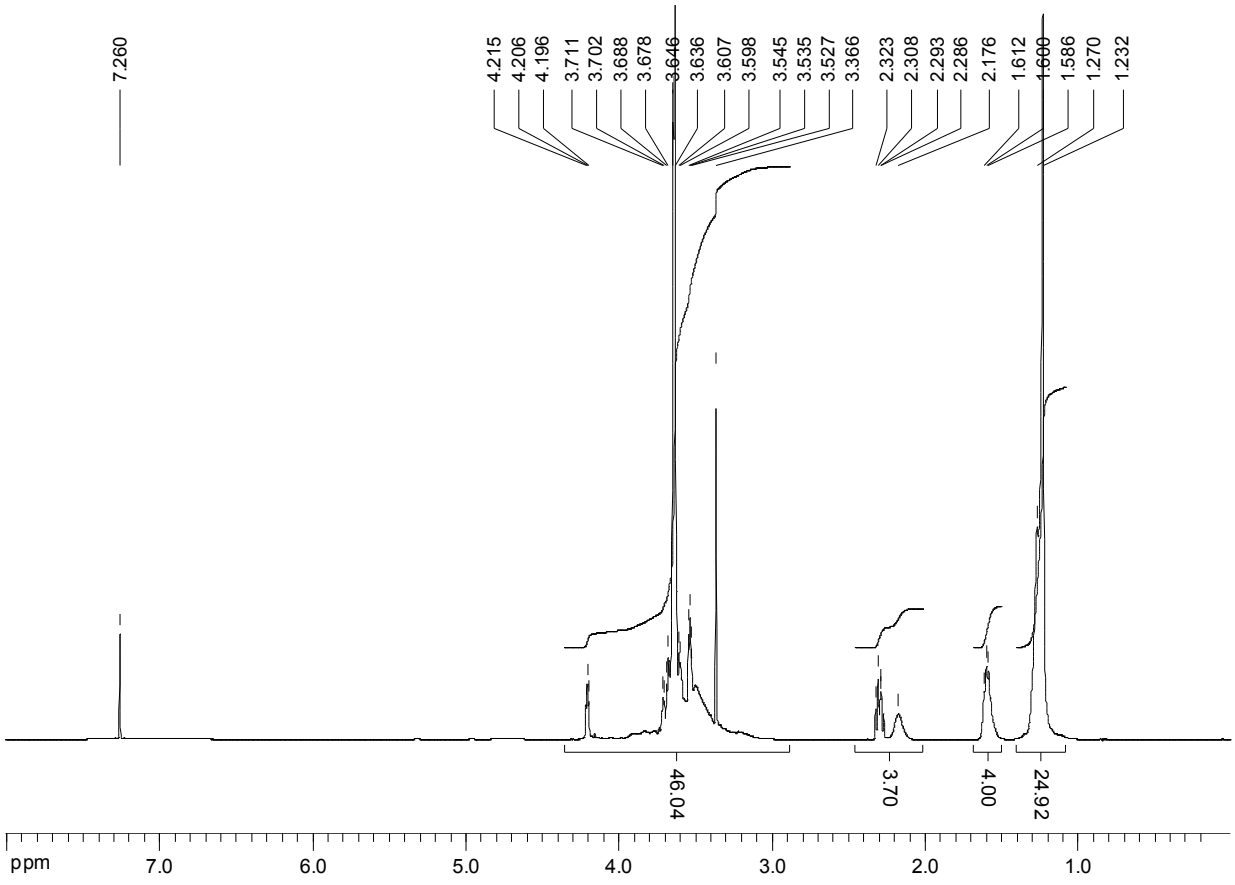


Figure 40. ^{1}H NMR spectrum of $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_6)_{1.0}$ in $CDCl_3$ as a typical spectrum of core-multishell architectures with PG-amine core. The polymer was obtained after reaction of $PG_{10000}(\text{-}NH_2)_{0.5}$ with $mPEG_6C_{18}ONSu$ active ester in MeOH for 24 h. The spectrum were recorded after dialysis in MeOH for 24 h. The peaks: at 1.10 – 1.35 ppm, multiplet at 1.50 – 1.70 ppm, triplet at 2.31 ppm and multiplet at 2.15 ppm belong to the aliphatic chain. The broad signal between 3.00 – 4.00 ppm belongs to the protons of PG-NH_2 core. Signals: singlet at 3.37 ppm, multiplets at 3.55 – 3.70 ppm and triplet at 4.20 ppm belongs to mPEG shell.

Table 10. Synthesized core-multishell architectures with PG-amine core. C_{18} was used as an inner shell for all polymers.

Polymer	M_n [g mol^{-1}]	Core	DF_{NH2}	DF_{PG}	mPEG
$PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_6)_{1.0}$	49000	$PG_{10000}(\text{-}NH_2)_{0.5}$	0.5	1.0	6
$PG_{10000}(\text{-}NH_2)_{0.7}(C_{18}mPEG_6)_{1.0}$	65000	$PG_{10000}(\text{-}NH_2)_{0.7}$	0.7	1.0	6
$PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_6)_{0.7}$	59000	$PG_{10000}(\text{-}NH_2)_{0.9}$	0.9	0.7	6
$PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_6)_{1.0}$	81000	$PG_{10000}(\text{-}NH_2)_{0.9}$	0.9	1.0	6
$PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_{14})_{1.0}$	75000	$PG_{10000}(\text{-}NH_2)_{0.5}$	0.5	1.0	14
$PG_{10000}(\text{-}NH_2)_{0.7}(C_{18}mPEG_{14})_{1.0}$	101000	$PG_{10000}(\text{-}NH_2)_{0.7}$	0.7	1.0	14
$PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_{14})_{0.7}$	91000	$PG_{10000}(\text{-}NH_2)_{0.9}$	0.9	0.7	14
$PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_{14})_{1.0}$	127000	$PG_{10000}(\text{-}NH_2)_{0.9}$	0.9	1.0	14

M_n = molecular weight of polymer calculated from the stoichiometrical amounts of PG-NH_2 and mPEG-C_{18}-ONSu applied for reaction; Core = type and functionalization of the core; DF_{NH2} = degree of PG amination calculated from ^{1}H NMR; DF_{PG} = degree of functionalization calculated from the stoichiometrical amounts of PG-NH_2 and mPEG-C_{18}-ONSu applied for reaction; mPEG = length of the poly(ethylene glycol) outer shell in average number of glycol units per chain.

4.3. Determination of the transport capacity of core-shell architectures with PG-amine core

The transport capacity of core-multishell architectures was determined by the solid-liquid extraction method. Congo red was used as a polar molecule. As an example for nonpolar guests nile red was chosen. Water was used as a non-solvent for nonpolar molecules and chloroform as a non-solvent for polar compounds. The encapsulation procedure was identical to the one described for PEI core polymers in chapter 3.4. The concentration of nanotransporters was 0.5 g l^{-1}. The TC was determined by UV/Vis spectroscopy.

The obtained results for TC for congo red (Figure 41; Table 11) revealed interesting transport – structure dependencies. First of all, as observed for PEI core polymers, the prolongation of the mPEG outer shell resulted in higher TC values. This tendency was independent of the functionalization level of the core. The improvement of the transport capacities lay between 1.5 for $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_x)_{1.0}$ and 1.9 times for $PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_x)_{1.0}$ with x = 6 or 14. The change of the shell density had no significant influence on the transport of congo red by PG-amine based nanotransporters. For polymers with the outer shell built of $mPEG_6$ all TC values oscillated in the range of 1.6 and 2.5 without showing a clear tendency. For polymers with longer mPEG chains the differences

between the transport capacities were even smaller (TC = 2.98 – 3.15) with only one exception. The polymer $PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_{14})_{1.0}$ revealed an approximately 35 % higher guest-host ratio (TC = 4.19). Additionally, polymers with free amine groups (core $PG_{10000}(\text{-}NH_2)_{0.9}$) revealed the lowest TC from all of the nanotransporters with PG core. However, these polymers were expected to possess the best transport efficiency due to ionic interactions between congo red and amine groups. Therefore it could be assumed that ionic interactions do not play the major role in the guest encapsulation by core-multishell architectures.

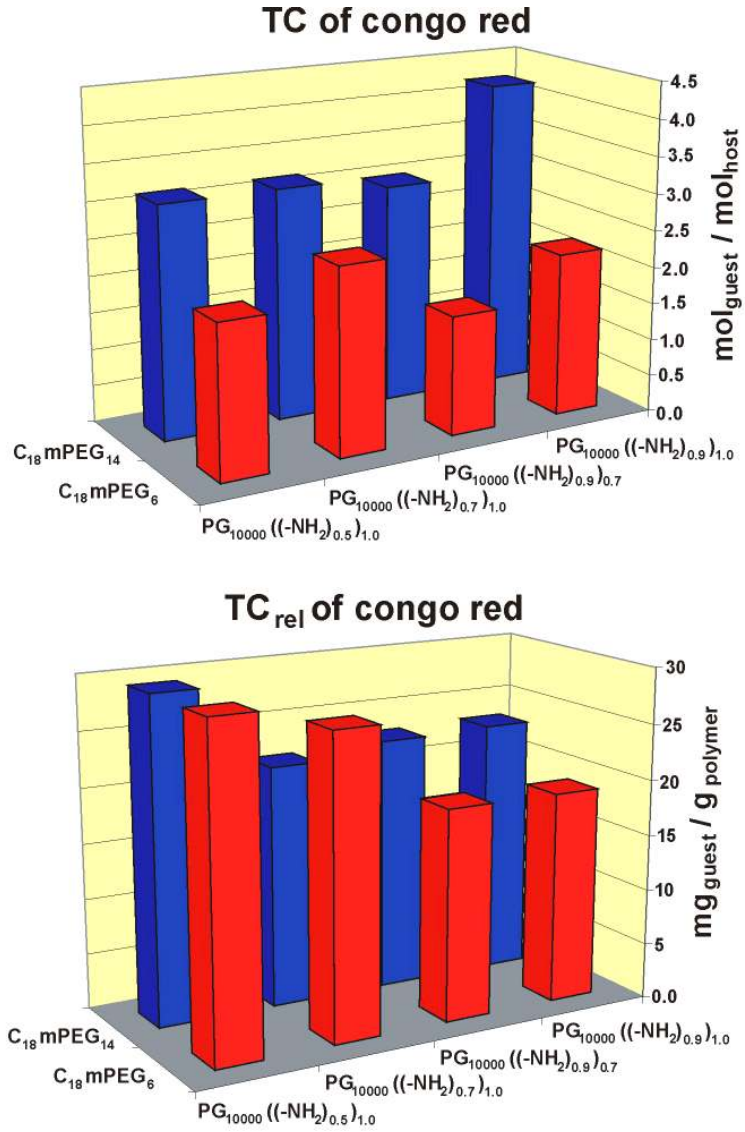


Figure 41. Transport capacity (top) and relative transport capacity (bottom) for congo red as an example of polar guest molecules with $PG_{10000}(\text{-}NH_2)_x(C_{18}mPEG_y)_z$ polymers; x = 0.5, 0.7, and 0.9; y = 6 (red), 14 (blue) and z = 0.7 or 1.0. For clarity error bars are not shown. The error is typically below 10 %.

Table 11. Transport capacity and relative transport capacity of core-multishell architectures with PG-amine core for congo red and nile red.

Polymer	M_n [g mol^{-1}]	congo red		nile red	
		TC[a]	TC_{rel}[b]	TC[a]	TC_{rel}[b]
$PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_6)_{1.0}$	49000	2.05	29.18	1.57	10.23
$PG_{10000}(\text{-}NH_2)_{0.7}(C_{18}mPEG_6)_{1.0}$	65000	2.52	27.00	1.82	8.91
$PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_6)_{0.7}$	59000	1.61	19.04	1.21	6.53
$PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_6)_{1.0}$	81000	2.21	18.98	1.71	6.72
$PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_{14})_{1.0}$	75000	3.15	29.22	2.50	10.63
$PG_{10000}(\text{-}NH_2)_{0.7}(C_{18}mPEG_{14})_{1.0}$	101000	3.15	21.71	3.82	12.04
$PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_{14})_{0.7}$	91000	2.98	22.82	1.90	6.65
$PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_{14})_{1.0}$	127000	4.19	23.03	1.87	4.69

Concentration of polymer was 0.5 g l^{-1}; M_n = molecular weight of polymer calculated from stoichiometrical amounts of PG-NH_2 and mPEG-C_{18}-ONSu applied; [a] transport capacity in mol_{guest} per mol_{host} ratio; [b] relative transport capacity in mg guest per g polymer; Error of TC is < 10 % for congo red and < 20 % for nile red.

The TC values suggested that polymers with the highest shell density and longest mPEG chains possess the best transport efficiency. The comparison of the relative transport capacities for all tested polymers revealed the opposite. With the increasing amount of amphiphilic chains from 64 ($PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_x)_{1.0}$) to 115 ($PG_{10000}(\text{-}NH_2)_{0.9}(C_{18}mPEG_x)_{1.0}$) a constant decrease of the transport efficiency (TC_{rel}) was observed. For polymers with $mPEG_6$ as outer shell the dropped of TC_{rel} by 45 % and for nanotransporters with $mPEG_{14}$ shell by 21 % was observed with increasing functionalization of the core.

The transport capacity tests performed for nile red revealed similar tendencies for TC and TC_{rel} (Figure 42; Table 11). First of all, an increase of the mPEG chain length resulted in a transport capacity improvement for nonpolar molecules. The TC change was more pronounced for polymers with lower core functionalization. For nanotransporters with a very dense shell the transport capacity only improved by 10 % between $mPEG_6$ and $mPEG_{14}$. An increase of the number of amphiphilic chains did not reveal any visible tendencies for the transport capacities of the polymers for both series ($mPEG_6$ and $mPEG_{14}$). Only for the $PG_{10000}(\text{-}NH_2)_{0.7}(C_{18}mPEG_{14})_{1.0}$ a very high TC value was observed.

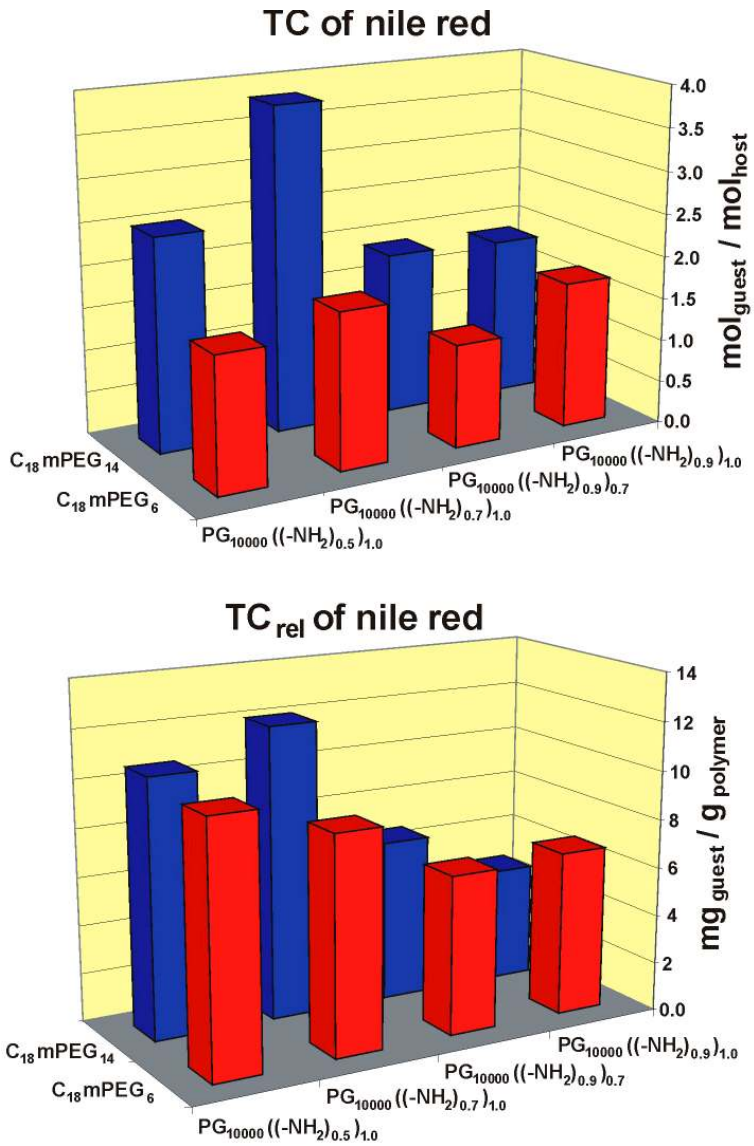


Figure 42. Transport capacity (top) and relative transport capacity (bottom) for nile red as an example of nonpolar guest molecules with $PG_{10000}(\text{-}NH_2)_x(C_{18}mPEG_y)_z$ polymers with x = 0.5, 0.7, and 0.9; y = 6 (red), 14 (blue) and z = 0.7 or 1.0. For clarity error bars are not shown. The error is typical below 20 %.

As observed for the encapsulation of congo red, the best relative transport capacity of nile red was obtained for the polymer with the lowest degree of functionalization. With increasing shell density a decrease of the transport efficiency was observed. In the polymer series with $mPEG_6$ shell TC_{rel} decreased by 33 % with increased the shell density. The TC_{rel} drop for the $mPEG_{14}$ polymer series was more significant and changed by 56 % due to increasing shell density.

The transport results obtained for polymers with PG-amine core are contradictory to expectation. Based on the results obtained for PEI core nanotransporters, a increasing core functionalization level should enhance the transport capacity for nonpolar molecules and should decrease it for polar ones. Meanwhile, the TC_{rel} for congo red and nile red revealed identical negative tendencies with increasing shell density. This is probably due to the

polarity differences between the PG-NH_2 and PEI cores. The polarity difference between amine group and amide bond is higher than between amide and hydroxyl groups. Therefore an increase of functionalization of the core for polymers with PG core does not influence strongly the polarity of the whole system and polymer polarity becomes unimportant for the TC dependency.

The influence of the polymer concentration on the transport capacity of core-multishell architectures with PG-NH_2 core was also determined. Tests were performed in chloroform with two polymers: $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_6)_{1.0}$ and $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_{14})_{1.0}$ as representatives. Congo red was used as a guest molecule. The polymer solutions were stirred for 24 h at r.t. with 20 mg of solid dye and then filtrated *via* 0.45 μm filters. As expected, the obtained results revealed a non-linear transport capacity-concentration dependency for both tested polymers (Figure 43). Below the concentration of 0.10 g l^{-1} (2.04×10^{-6} M for $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_6)_{1.0}$ and 1.33×10^{-6} M for $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_{14})_{1.0}$) no encapsulation of congo red was observed including UV/Vis spectroscopy. At the concentration of 0.10 g l^{-1} for both polymers a very limited transport capacity was observed. At the concentration of 0.20 g l^{-1} and higher, nanotransporters with PG-amine core revealed very good transport efficiencies. The maxima of the TC were located at the threshold concentration (0.20 g l^{-1}) independent of the polymer. This behavior, with a clear point of the critical aggregation concentration (CAC), is identical with the one observed for the polymers with PEI core. Therefore it could be assumed that in both cases (PEI and PG-amine core) the identical mechanism of guest encapsulation *via* aggregate formation is responsible for the host-guest interactions. In contrast to the PEI core polymers with CAC in the range of 0.05 – 0.10 g l^{-1}, PG-amine core-shell structures aggregate at slightly higher concentration of 0.20 g l^{-1}. However, the beginning of the aggregation process could be observed at the concentration of 0.10 g l^{-1}. The self-assembly of the polymers into supramolecular aggregates was confirmed by dynamic light scattering (see chapter 6).

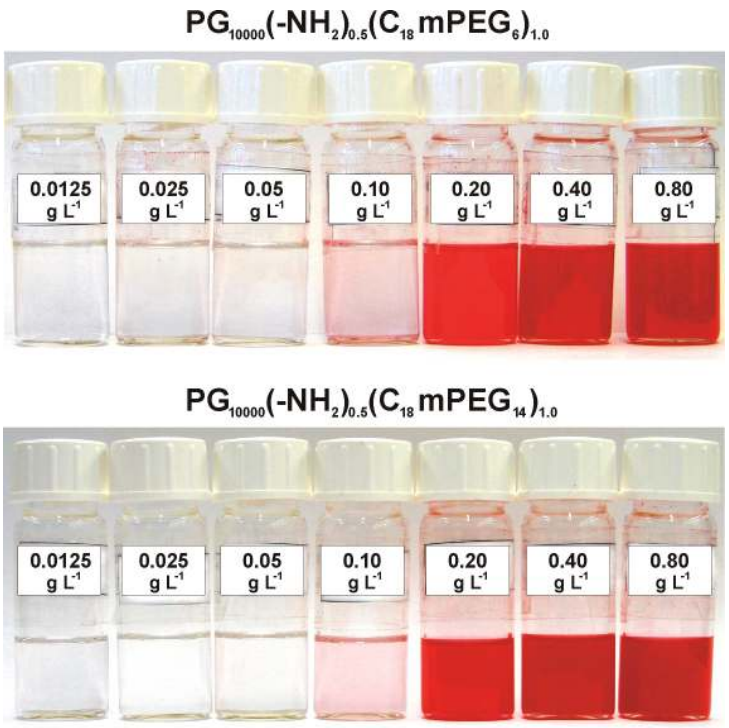


Figure 43. Influence of the polymer concentration on the transport capacity of polymers: $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_6)_{1.0}$ (top) and $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_{14})_{1.0}$ (bottom) for congo red. Samples were prepared by stirring the polymer solutions with 20 mg of solid dye in chloroform for 24 h at r.t. Then the samples were filtrated *via* 0.45 μm PTFE filters. In both cases the critical aggregation concentration (CAC) point is clearly visible at the concentration of 0.20 g l^{-1}. Some encapsulation of congo red can be detected at conc. 0.10 g l^{-1}.

Measurement of the encapsulation dynamics were performed for $PG_{10000}(\text{-}NH_2)_{0.7}(C_{18}mPEG_6)_{1.0}$ at the concentration of 0.5 g l^{-1} (7.69×10^{-6} M). As a guest molecule nile red was used and 5 mg of the solid dye were added to 4 ml of the polymer solution in water. The results revealed a similar encapsulation-time dependency as observed for polymers with the PEI core. In the first 3 h of the experiment very fast growth of the TC value was observed which slowed down after 24 h of stirring. At this point the TC was equal to 3.4. Slower but almost constant growth in transport capacity was observed with stirring longer than 24 h. The ratio of 12.8 guest molecules per host was obtained after 315 h of the experiment. Thus, the TC increased 3.8 times between the 24th and 315th hour of the encapsulation process. No saturation point of the polymer was detected. In comparison to $PEI_{10500}(C_{18}mPEG_6)_{0.7}$ the obtained TC ratio was higher by a factor of 2.5 after 24 h of stirring and by a factor of 3.5 after 168 h of experiment. Also the increase of the transport capacity after one day of stirring was faster for the polymer with PG-amine core.

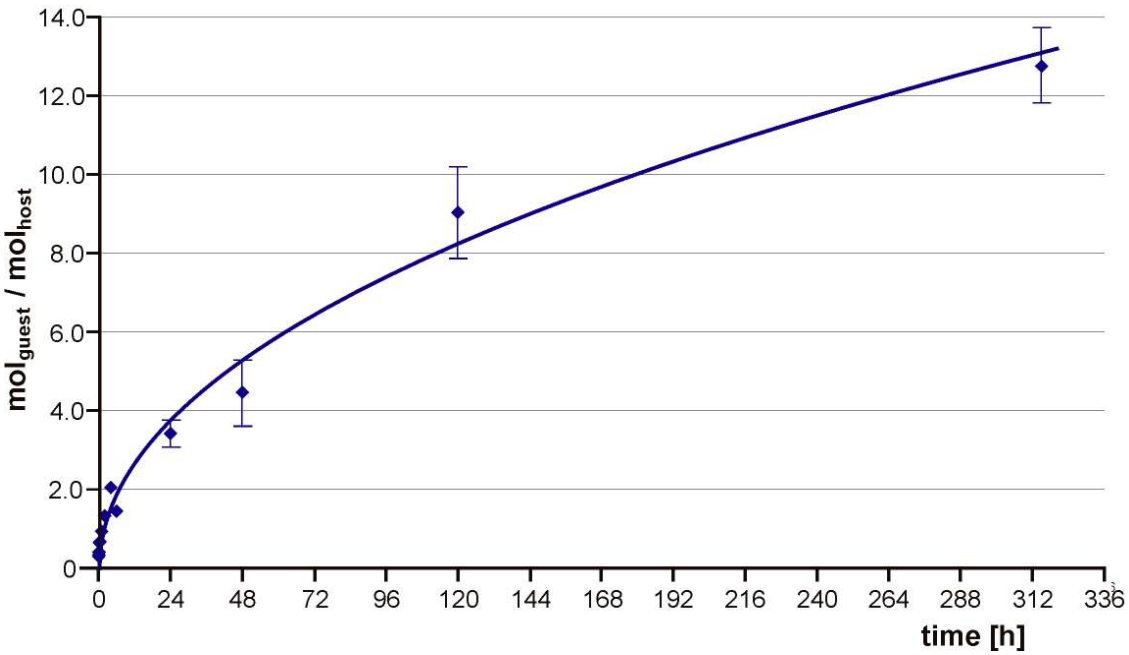


Figure 44. The dynamics of nile red encapsulation by $PG_{10000}(\text{-}NH_2)_{0.7}(C_{18}mPEG_6)_{1.0}$. Concentration of polymer = 0.5 g l^{-1} (7.69×10^{-6} M). 4.0 ml of polymer solution and 5 mg of dye were used in the solid-liquid extraction method.

In summary, core-multishell architectures with polyglycerylamine core revealed similar encapsulation abilities as polymers with PEI core. The universal transport abilities were confirmed by the encapsulation of congo red and nile red as representatives for polar and nonpolar guest molecules, respectively. Also the self-assembly of core-shell nanotransporters into aggregates at similar CACs was confirmed. The responsibility of supramolecular aggregates for the host-guest ability was confirmed. The obtained TC and TC_{rel} values for those polymers were lower than the ones measured for PEI based systems. Although, after longer times of the encapsulation experiment the transport efficiency was more than 2 times better for the PG-amine core polymers than for PEI core nanotransporters. This is probably due to the much higher functionalization level of this new type of polymers which can inhibit the encapsulation dynamics due to the greater density of the shell that results in a worse intramolecular cavities accessibility. In the case of nonpolar molecules no overloading of the nanotransporters was detected. However, the encapsulation experiment with rose bengal revealed a decrease of the TC with a increasing amount of solid dye (rose bengal) (Figure 45). The results were similar to those obtained for polymers with PEI core.

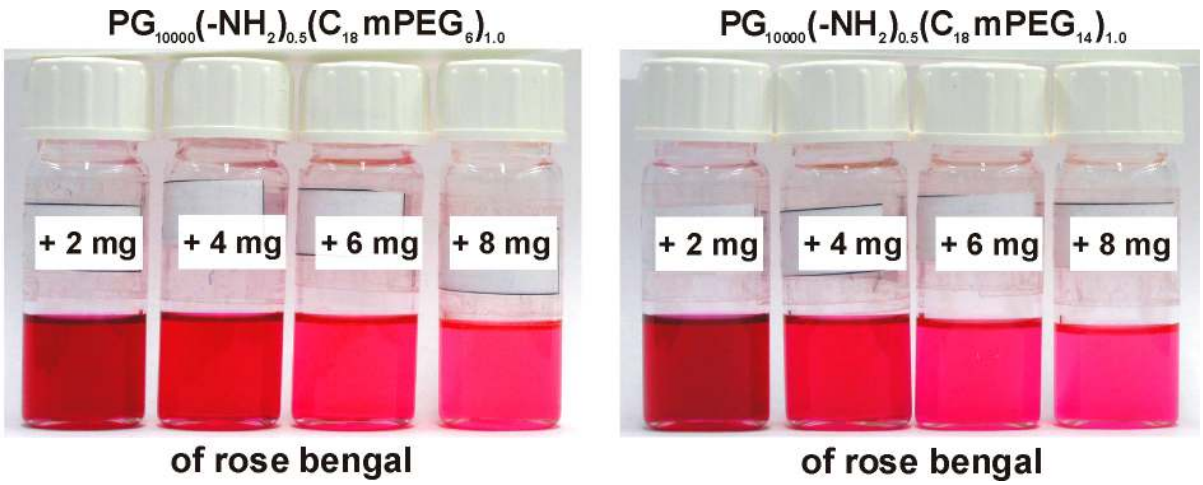


Figure 45. The transport capacity of $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_6)_{1.0}$ (left) and $PG_{10000}(\text{-}NH_2)_{0.5}(C_{18}mPEG_{14})_{1.0}$ (right) for rose bengal obtained by addition of 2, 4, 6, and 8 mg of solid dye to 4 ml of the polymer chloroform solution (concentration 0.5 g l^{-1}; 1.02 $\times$ 10^{-5} M and 6.67 $\times$ 10^{-6} M). Samples were stirred for 24 h at r.t. and filtrated *via* 0.45 μm filter. The change of the color from dark red to light pink indicates the decrease of the TC. This is due to precipitation of the polymer as a result of its oversaturation with the dye.