Supporting Information

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pH-Responsive Molecular Nanocarriers Based on Dendritic Core-Shell-Architectures

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**Typical procedure for transketalisation reaction of polyglycerol (1)**

To a solution of 15 mL acetone dimethylacetal (122.2 mmol) and 100 mg p-toluene sulfonic acid (PTSA) as catalyst 2.5 g polyglycerol ($M_n = 21,000$ g mol$^{-1}$, PD = 1.7, 13.5 mmol OH per g, 8.1 mmol diols per g) were added under argon. The reaction was performed under ultrasonication over four hours at 70 °C. Subsequently all volatile materials were removed under reduced pressure to obtain the PG-acetoneketal$^{[21]}$ as yellow oil, which was directly used in the second transketalisation reaction.

To a solution of 2.9 g PG-acetoneketal (3.45 mmol acetal groups per g) in 50 mL abs. toluene a solution of 14.15 mmol (1.3 eq.) of the carbonyl compound (C$_{33}$-ketone) in 50 mL abs. toluene was added. The mixture was heated up to 125 °C oil bath temperature for 8 hours and most of the toluene was distilled off in order to remove acetone residues freed from the reaction. Another 100 mL abs. toluene were slowly added under distillative conditions during the reaction in order to avoid dry up of the reaction mixture. After cooling to room temperature 1 mL (10 mmol) of piperidine was added to neutralize the remaining PTSA. The solvent was evaporated and dialysis in chloroform using a benzyolated cellulose membrane (MWCO 1000, Sigma) was performed for two days in order to remove traces of PTSA, piperidine and unreacted carbonyl compound. The purified product was dried under vacuum to obtain 5.2 g (73%) of a white solid 3b.

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ (ppm) = 0.8 – 0.9 [t, PG-C(CH$_2$(CH$_2$)$_{14}$CH$_3$)$_2$], 1.1 – 1.3 [m, PG-C(CH$_2$(CH$_2$)$_{14}$CH$_3$)$_2$], 1.5 – 1.6 [m, PG-C(CH$_2$(CH$_2$)$_{14}$CH$_3$)$_2$], 3.2 – 4.3 [m, PG-C(CH$_2$(CH$_2$)$_{14}$CH$_3$)$_2$]; $^{13}$C-NMR (CDCl$_3$, 75.4 MHz): $\delta$ (ppm) = 14.1 [PG-C(CH$_2$CH$_2$(CH$_2$)$_{11}$CH$_2$CH$_2$CH$_3$)$_2$], 22.7 [PG-C(CH$_2$CH$_2$(CH$_2$)$_{11}$CH$_2$CH$_2$CH$_3$)$_2$], 23.9 [PG-C(CH$_2$CH$_2$(CH$_2$)$_{11}$CH$_2$CH$_2$CH$_3$)$_2$], 29.7 [PG-C(CH$_2$CH$_2$(CH$_2$)$_{11}$CH$_2$CH$_2$CH$_3$)$_2$], 31.9 [PG-C(CH$_2$CH$_2$(CH$_2$)$_{11}$CH$_2$CH$_2$CH$_3$)$_2$], 37.5 [PG-C(CH$_2$CH$_2$(CH$_2$)$_{11}$CH$_2$CH$_2$CH$_3$)$_2$], 60–80 [PG-C(CH$_2$CH$_2$(CH$_2$)$_{11}$CH$_2$CH$_2$CH$_3$)$_2$], 112.8 [PG-C(CH$_2$CH$_2$(CH$_2$)$_{11}$CH$_2$CH$_2$CH$_3$)$_2$]
\( ^1 \text{H-NMR of 3b} \)

**Calculation of the reaction ratio**

Signal 1.5 – 1.6 ppm: 4 hydrogen atoms \( \rightarrow \) 1 hydrogen atom: \( I = 0.25 \)

Signal 3.2 – 4.4 ppm: 5 hydrogen atoms \( \rightarrow \) 1 hydrogen atom: \( I = \frac{5.452}{5} = 1.09 \)

Relative number of reacted monomer units: \( \frac{0.25}{1.09} = 22.9 \% \rightarrow 45.8 \% \) of the OH groups have reacted (45.8 \% = degree of alkylation)

\( \Rightarrow \) 30 \% of the monomer units are terminal units (60 \% OH groups)

\( \Rightarrow \) \( \frac{22.9}{30} = 76 \% \) conversion
$^{13}$C-NMR of 3b
Typical procedure for the preparation of PEI–Imine (4b)

To a heterogeneous mixture of 3.5 g PEI$_{581}$ ($M_n = 25000$ g mol$^{-1}$, PD = 2.5, 26.6 mmol terminal NH$_2$ units) in 100 mL toluene 4.53 g (26.6 mmol) 6-undecanone were added under nitrogen atmosphere. The mixture was refluxed for 48 hours under dean-stark conditions and was clear at the end of the reaction. After cooling to room temperature the solvent was evaporated and the polymer was dried under vacuum to obtain 7.8 g of a pale yellow oil of the PEI-imine 4b. Integration of $^1$H-NMR signals indicates 82 % conversion of the terminal groups.

IR (KBr): $\tilde{\nu} = 1655$ cm$^{-1}$ (C=N)

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta = 0.78$ (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 1.17 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 1.36 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 2.09 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 2.3 – 2.8 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 3.28 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$)

$^{13}$C-NMR (CDCl$_3$, 75.4 MHz): $\delta = 13.9$ (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 22.4 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 26.3 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 31.6 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 40.1 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 45 – 55 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$), 174.1 (PEI-CH$_2$NC(CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$)$_2$)
**Calculation of terminal units conversion ratio from $^1$H-NMR**

Signal 3.2 – 3.4 ppm: 2 hydrogen atoms -> 1 hydrogen atom: $I = 0.5$

PEI-core: 2.3 – 3.4 ppm: 4 hydrogen atoms -> 1 hydrogen atom: $I = (6.473 + 1)/4 = 1.87$

Conversion ratio = $0.5/1.87 = 26.7\%$ of all monomer units

- $0.267 \times 581 = 155$ units have reacted (number of terminal units in PEI$_{581} = 190$)
- conversion ratio = $155/190 = 82\%$ (82\% of terminal units have reacted)
- degree of alkylation = 53%
\(^{13}\)C-NMR of 4b
Number of terminal units in polyethylenimine 2 (PEI₅₈₁, 25000 g/mol) determined with inverse gated $^{13}$C-NMR in CDCl₃ + Cr(AcAc)₃

$$T = \text{terminal units}, \quad L = \text{linear units}, \quad D = \text{dendritic units}$$

$$a = c, \quad T = a + c + 2b, \quad T = D, \quad DB = \frac{2 \cdot T}{\sum \text{Integrals}} = 65\%$$

PEI₅₈₁ has 190 terminal, 188 dendritic and 203 linear nitrogen atoms
**Determination of transport capacities for nanocarrier 3c:**

In order to determine the different transport capacities of these dendritic core-shell polymers, the water-soluble dye congo red was investigated as an anionic model compound, and chloroform as organic phase was used, in which this dye is not soluble. In a typical experiment 3 mL of the respective aqueous dye solution was manually shaken for some seconds with 3 mL chloroform solution of the shell-functionalized polyglycerol/polyethyleneimine 3.4. Only the concentration of the dye in water was changed, the concentration of the polymer in chloroform remains unchanged. After phase separation, 2 mL of the organic layer were transferred into a UV/VIS cuvette and measured (Figure 1). The absorption maximum at 345 nm was plotted versus the concentration (Figure 2). In all cases, a linear increase of the colour intensity of the organic phase is observed below the saturation point. Once the saturation concentration of the dye in the organic phase is reached, the absorption remains nearly the same with further increase of the dye concentration in the aqueous phase (Figure 2). At dye concentrations below the saturation concentration congo red was quantitatively extracted from the aqueous layer into chloroform. Alternatively the aqueous phase could also be investigated with UV/VIS (Figure 3) leading to similar results. The solutions remained unchanged over several months.

The average maximum load of dye molecules per polymer can be calculated from these UV/VIS experiments. The determined saturation concentration of the dye in the aqueous phase has to be divided with the concentration of the polymer in the chloroform phase which is constant (Figure 4).

In the case of 4b the transport (loading) capacities can also be determined by dissolving the guest molecule in the organic solution of the nanocarrier (chloroform, toluene) and subsequent UV measurements.
**Figure 1.** UV/VIS measurement of nanocarrier 3c in chloroform (60.3*10^{-6} mol/l) with encapsulated dye congo red using different concentrations of dye congo red in aqueous phase see legend of graph.

![UV/VIS measurement graph](image)

**Figure 2.** Determination of saturation concentration [dye] from UV/VIS absorbance of the chloroform phase. Concentration of nanocarrier 3c: 60.3*10^{-6} mol/l.

![Saturation concentration graph](image)
**Figure 3.** Determination of saturation concentration [dye] from UV/VIS absorbance of the aqueous phase. Concentration of nanocarrier 3c: 60.3\(\times 10^{-6}\) mol/l.

**Figure 4.** Determination of the transport capacity \(n\)

\[ n = \frac{[\text{dye}]}{[\text{polymer}]} \]
**Figure A.** Encapsulation and transport of different dye structures from aqueous phase to chloroform phase using the nanocarriers 3b or 4a. Colourless aqueous phases demonstrate the complete transport upon encapsulation (left pictures) and colourless organic phases show the insolubility of dyes without nanocarriers (right pictures).

**Figure B.** Addition of aqueous congo red solution to the dissolved nanocarrier 3b in chloroform with encapsulated bromophenol blue. Weaker binding guest (bromophenol blue) is replaced with congo red, which has a higher binding affinity.
Dialysis experiments of dye (sodium pikrate) in the presence of unfunctionalized dendritic polymers

Dialysis tubing (MWCO 1000 g/mol, benzylated cellulose, Sigma) was used in water.
Dialysis of PG 1 with sodium pikrate (pH 7): Pikrate is release from the dialysis tube.
Dialysis of PEI 2 with sodium pikrate (buffer solution: pH=6, pH=7, pH=8, without buffer: pH=10): Pikrat is released from the dialysis tube if buffer solution is used. Pikrat is complexed to a small extent without buffer solution.
Dialysis with congo red was not successful due to complexation of congo red with dialysis membrane (congo red stays inside even without polymer).

Figure C. $^1$H-NMR spectra (300 MHz, CDCl$_3$): upper spectrum: complex of the hydrophilic dye methyl-red and the amphiphilic PG-ketal 3b; bottom spectrum: saturated solution of methyl-red in chloroform. Line broadening and shift of signals (i.e. methyl groups) can be observed upon encapsulation.
Figure D. AFM-image of the PEI-imine 4a on a mica surface. (PEI: $M_n = 25000$g/mol)

Figure E. AFM-image of the PEI-imines 4b on a mica surface. Aggregate formation might be due to partial hydrolysis on the mica surface. (PEI: $M_n = 25000$g/mol)