Supporting Information

Structure-activity Relationship Study of Dendritic Polyglycerolamine for Efficient siRNA Transfection

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1. Ethidium Bromide displacement assay

![Figure 1. EthBr displacement assay. Fluorescence intensity versus N/P ratios of different dPG-NH₂ analogues.](image)

2. Gel Electrophoresis

The ability of dPG-NH₂ analogues to form polyplexes with free siRNA was analyzed by electrophoretic mobility shift assay. Therefore, stock solutions of dPG-NH₂ 90% (1 mg in 5 mL ultrapure water, 2.48 mM amine) and dPG-NH₂ 10% (1 mg in 5 mL of the same solvent, 0.28 mM amine) were prepared. Different quantity of dPG-NH₂ solutions were mixed with 3.3 µL (5 nmol phosphate) of a FECH siRNA solution (40 µM siRNA) in ultrapure water to achieve different N/P ratios. The mixtures were incubated at rt for 30 min and then were applied to gel electrophoresis (for 35 min, 50 V) The agarose gel was stained with ethidium bromide for visualization.
Figure 2. Agarose gel electrophoresis retardation assay of dPG-NH$_2$ 90%/siRNA polyplexes at different N/P ratios (left side) and dPG-NH$_2$ 10%/siRNA polyplexes (right side). In each case free siRNA appears in the second lane from the right and the reference appears in the middle.

Figure 3. Left: 2D drawing of the molecular structure used to build the 3D model of the dPG-NH$_2$ molecules showing dPG-NH$_2$ 10% as an example. Right: 21-bp DNA 3D model used in all simulations. The sequence is identical to those experimentally employed (color code: blue, adenine; orange, cytosine; red, guanosine; cyan: thymine; see also main text for more details).

3. Detailed synthetic procedure of dPG-NH$_2$ analogues of different DF and NMR spectras

To convert 10, 30, 50, and 90% of all hydroxyl groups on dPG into amines, the hydroxyl groups were first activated using methanesulfonyl chloride. Different DF on dPG were achieved by controlling the degree of mesylation in the first synthetic step. For converting 10% of all hydroxyl groups on a 10 kDa dPG, first 1 g (0.1 mmol, 14 mmol OH) dPG was dried and then dissolved in 8 mL abs. pyridine. This synthetic step was conducted under inert gas atmosphere and exclusion of water. The reaction mixture was cooled down to 0 °C using NaCl/ice bath. Meanwhile a solution of methanesulfonyl chloride (0.24 g, 2.1 mmol)
in 2 mL abs. pyridine was added dropwise to the reaction mixture. The resulting brown mixture stirred under this condition for 16-24 h (depending on the functionalization degree). Upon addition of ice to the reaction mixture a brown solid precipitated which was then washed with water three times. The mesylated dPG was dissolved and dialyzed extensively in methanol (or a mixture of methanol with aceton according to the DF) to yield a brownish honey-like product.

**Scheme 1.** Three step synthesis of dPG-NH$_2$. Mesylation of hydroxyl groups on dPG followed by azide substitution. In the last step azides were reduced to amines by Staudinger reaction.

The degree of functionalization was assigned by comparing the corresponding signal of methyl group of mesylate in $^1$HNMR with the dPG back bone signal. The corresponding $^1$HNMRs of dPG-OMs 10% and dPG-OMs 90% for comparison are shown below. $^1$H-NMR of dPG-OMs 10% (400 MHz, (CD3OD): $\delta =$ 4.6 – 3.4 (dPG backbone), 3.28 (CH$_3$), 0.9 (PG-starter). In the next step, O-Mesyl groups on dPG were substituted by azides. In a single-neck flask equipped with a reflux condenser and stirrer, dPG with various mesylation degree was dissolved in DMF. According to the DF, different quantity of NaN$_3$ was added to this mixture. The suspension was then stirred up to 3 days at 60 °C. Disappearance of the methyl signal was confirmed by $^1$H NMR spectroscopy. Upon azidation the corresponding signal of azide functionality appeared at (2100 cm$^{-1}$). The disappearance of methyl group in $^1$HNMR and the appearance of the azide signal in IR spectroscopy confirmed the substitution of mesyl groups by azides. Finally, the reduction of the azides by triphenylphosphine via Staudinger reduction resulted in the formation of dPG with various amine DF (Scheme 1). The reduction of azides to amines was examined by
$^1$HNMR and IR spectroscopy. For optimal purification, extensive dialysis was carried out after each reaction step.

$^1$H-NMR Spectrum of Mesylated dPG (dPG-OMs 10 and 90%)
$^1$H-NMR Spectrum of dPG-NH$_2$ analogues

dPG-NH$_2$ 10%  


dPG-NH$_2$ 30%
13C-NMR Spectrum of dPG

Equation of degree of branching:  \( DB = \frac{2D}{2D + L} \)

D: Dendritic units  
L: Linear groups

4. Size distribution of dPG-NH₂ analogues by intensity and number

dPG-NH₂ 10%
dPG-NH$_2$ 30%

dPG-NH$_2$ 50%
5. Computational Details

**MM/PBSA calculations.** The free energy of binding between the dPG-NH$_2$s and the DNA was calculated according to a previously validated approach based on the Molecular Mechanics/Poisson–Boltzmann surface area (MM/PBSA) methodology.
This computational technique employs snapshots taken from MD trajectories to estimate the average interaction energies based on the solute molecular mechanics internal energy ($\Delta E_{\text{MM}}$) and solvation energy ($\Delta G_{\text{solv}}$), this last obtained via Poisson-Boltzmann (PB) continuum solvent calculations. According to MM/PBSA, the overall binding energy $\Delta G_{\text{bind}}$ is given by the difference in energy between the dPG-NH$_2$/DNA complex and the individual polyamine and nucleic acid:

$$\Delta G_{\text{bind}} = \Delta G_{\text{dPG-NH}_2/DNA} - \Delta G_{\text{dPG-NH}_2} - \Delta G_{\text{DNA}}$$  \hspace{1cm} (1)

where:

$$\Delta G_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} - T\Delta S$$  \hspace{1cm} (2)

$$\Delta E_{\text{MM}} = \Delta E_{\text{int}} + \Delta G_{\text{vdW}} + \Delta E_{\text{ele}}$$  \hspace{1cm} (3)

$$\Delta E_{\text{int}} = \Delta E_{\text{bond}} + \Delta E_{\text{angle}} + \Delta E_{\text{tors}}$$  \hspace{1cm} (4)

$$\Delta G_{\text{solv}} = \Delta G_{\text{PB}} + \Delta G_{\text{np}}$$  \hspace{1cm} (5)

$\Delta E_{\text{MM}}$ is the system change in molecular mechanical energy upon binding, which consists of internal energy $\Delta E_{\text{int}}$ (due to bonds, angles, and dihedral angles variations) electrostatic energy ($\Delta E_{\text{ele}}$) and van der Waals ($\Delta E_{\text{vdW}}$) contributions. The solvation energy term $\Delta G_{\text{solv}}$ consists of two components: the electrostatic term $\Delta G_{\text{PB}}$ and the nonpolar term $\Delta G_{\text{np}}$, respectively. $\Delta G_{\text{PB}}$ is obtained by solving the Poisson-Boltzmann equation while $\Delta G_{\text{np}}$ can be obtained via the semiempirical expression: $\Delta G_{\text{np}} = \gamma \times \text{SASA} + \beta$, in which SASA is the solvent accessible surface area of the molecule, $\gamma$ is the surface tension parameter (0.00542 kcal/Å$^2$/mol), and $\beta = 0.92$ kcal/mol. Finally, the entropic contribution $-T\Delta S$ is calculated via normal mode of harmonic frequencies obtained from a subset of minimized snapshots taken from the corresponding MD trajectories.