Supporting Information

Hyperbranched PEI with various oligosaccharide architectures:
Synthesis, characterization, ATP complexation and cellular uptake properties

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Calculation of the degree of functionalization (DF) and total degree of functionalization (TDF) of modified PEI based on PEI-II from elemental analysis

Example for 2-Mal:

Elemental analysis:  C = 44.42 %, N = 3.94 %, H = 7.19

DP = 84, a = number of coupled maltose

\[ \text{M}_{\text{Polymer}} = (\text{C}_2\text{H}_5\text{N}) \times 84 + a \times (\text{C}_{12}\text{H}_{23}\text{O}_{10}) \]

Nitrogen content: \[ N = \frac{84 \times 14}{\text{M}_{\text{Polymer}}} \]
Carbon content: \[ C = \frac{(2 \times 12 \times 84 + a \times 12 \times 12)}{\text{M}_{\text{Polymer}}} \]

N/C ratio is 3.94 / 44.42, a = 77.7

The degree of functionalization (DF) based on the conversion of twice T units and one L unit: \[ \frac{77.7}{(2 \times 27.4 + 30.8)} = 91 \% \]

The degree of total functionalisation (TDF) based on the conversion of all branching units in the PEI derivative (twice T units, one L unit and one D unit): \[ \frac{77.7}{(2 \times 27.4 + 30.8 + 25.8)} = 70 \% \]

Calculation of the degree of branching units (T, L or D units) of modified PEI based on PEI-II from elemental analysis

Example for 2-Mal:

Number of coupled maltose units (a) on PEI-II is 77.7 received from the calculation of DF. PEI-II possesses 27.4 T units, 30.8 L units and 25.8 D units (Table 3) at which twice conversion of T units is possible finally to result into D units.

The calculation bases on the assumption that at first the T units are converted into L units and then the L units can be converted into D units. Therefore, 27.4 T units are converted into 27.4 L units. From all L units (\( \Sigma 58.2 \)) 50.3 units are also converted into 50.3 D units. The final calculation gave that 7.9 L units (9.4 %) and 76.1 D units (90.6 %) are present in 2-Mal-I.

Calculation of the molecular weight (\( M_n \)) for the PEI derivative used in the ITC study

Need of DP and \( M_n \) of the parent PEI (PEI-II and PEI-II) which is presented in Table 3. Need of number of chemically coupled oligosaccharide received by calculation of the degree of functionalization (DF) from elemental analysis.

Example for 4-Mal:

\[ \text{DP} = 84, \text{ } M_n = 3600 \text{ g/mol}, a = \text{number of coupled maltose} \]

\[ \text{M}_{\text{Polymer}} = (\text{C}_2\text{H}_5\text{N}) \times 84 + a \times (\text{C}_{12}\text{H}_{23}\text{O}_{10}) \]

Nitrogen content: \[ N = \frac{84 \times 14}{\text{M}_{\text{Polymer}}} \]
Carbon content: \[ C = \frac{(2 \times 12 \times 84 + a \times 12 \times 12)}{\text{M}_{\text{Polymer}}} \]
N/C ratio is 8.09 / 44.53, a = 31

Then, determination of $M_n$ of chemically coupled maltose unit on PEI-core with $a = 31$. This means the calculation of $31 \times (C_{12}H_{23}O_{10})$ followed by the addition of $M_{n,PEI}$ and $M_{maltose}$. Thus, the sum of $M_n$ is 13800 g/mol for 4-Mal.
Figure Caption for Supporting Information

**Figure 1-SI** $^1$H spectra of 1-Mal-III and 5-Mal-III obtained from substrate ratio PEI-I/Mal-III 1:5 and PEI-III/Mal-III 1:2, respectively.

**Figure 2-SI** $^1$H NMR spectrum of 2-Lac obtained from substrate ratio PEI-II/Lac 1:5.

**Figure 3-SI** $^1$H spectra of 4-Mal and 6-Mal obtained from substrate ratio PEI-II/Mal 1:0.5 and 1:0.2, respectively.

**Figure 4-SI** $^1$H NMR spectrum of 6-Mal-VII based on the substrate ratio PEI-II/Mal-VII 1:0.5 (R = reductively coupled maltoheptaose unit).

**Figure 5-SI** $^{13}$C NMR spectra of (A) 1-Mal-III based on the substrate ratio PEI-I/Mal-III 1:5 and (B) 3-Mal-III based on the educt ratio PEI-III/Mal-III 1:2.

**Figure 6-SI** $^{13}$C NMR spectra of 2-Mal obtained from substrate ratio PEI-II/Mal 1:2 and 1:10, respectively.

**Figure 7-SI** $^{13}$C NMR spectrum of 2-Lac and 4-Lac based on the substrate ratio PEI-II/Lac 1:5 and 1:0.4, respectively.

**Figure 8-SI** $^{13}$C NMR spectra of 2-Mal-III based on the substrate ratio PEI-II/Mal-III 1:5.

**Figure 9-SI.** $^{13}$C NMR spectra of (A) 4-Mal-III based on the substrate ratio PEI-II/Mal-III 1:0.5

**Figure 10-SI** $^{13}$C NMR spectrum of 6-Mal-VII based on the educt ratio PEI-II/Mal-VII 1:0.5 (R = reductively coupled maltoheptaose unit).

**Figure 11-SI** ATR-IR spectrum of PEI-II.
Figure 12-SI  ATR-IR spectrum of 2-Glc with structure A.

Figure 13-SI  ATR-IR spectrum of 2-Mal with structure A.

Figure 14-SI  ATR-IR spectrum of 5-Mal-III with structure B.

Figure 15-SI  ATR-IR spectrum of 4-Mal with structure B.

Figure 16-SI  ATR-IR spectrum of 2-Lac with structure A.

Figure 17-SI  Binding of ATP to PEI-III and 3-Mal-III and 7-Mal-III which possess PEI-III as core molecule.

Figure 18-SI  -fold increase in nucleotide uptake upon complexation (HepG2 cells): procedure as mentioned for Figure 8.

Table 1-SI  Influence of the substrate ratio PEI-II : oligosaccharide (OS) and PEI-III : OS on the degree of functionalization (DF), total degree of functionalization (TDF) of modified PEI and the determination of the degree of T, L and D units obtained from elemental analysis.

Table 2-SI  Comparison of $^{13}$C chemical shifts of D, L and T units for PEI-II and PEI-III and (oligo-)saccharide-modified PEI based on modified PEI-II and PEI-III in D$_2$O.

Table 3-SI  $^{13}$C signal assignment for PEI-bonded glucose (Glc), maltose (Mal) and maltotriose (Mal-III)
Figure 1-SI  $^1$H spectra of 1-Mal-III with structure A (top) and 3-Mal-III with structure B (bottom) obtained from educt ratio PEI-I/Mal-III 1 : 5 and 1 : 2, respectively.
Figure 2-SI $^1$H NMR spectrum of 2-Lac with structure A obtained from educt ratio PEI-II/Lac 1:5.
Figure 3-SI  $^1$H spectra of 4-Mal with structure B (top) and 6-Mal with structure C (bottom) obtained from educt ratio PEI-II/Mal 1:0.5 and 1:0.2, respectively.
Figure 4-SI $^1$H NMR spectrum of 6-Mal-VII with structure C based on the educt ratio PEI-II/Mal-VII 1 : 0.5 (R = reductively coupled maltoheptaose unit; Scheme 1).
Figure 5-SI $^{13}$C NMR spectra of 1-Mal-III with structure A (top) based on the educt ratio PEI-I/Mal-III 1 : 5 and 3-Mal-III with transition from structure A to B (bottom) based on the educt ratio PEI-III/Mal-III 1 : 2.
Figure 6-SI  $^{13}$C NMR spectra of 2-Mal obtained from educt ratio PEI-II/Mal 1 : 2 and 1 : 10, respectively.
Figure 7-SI  $^{13}$C NMR spectrum of 2-Lac with structure A (top) and 6-Lac with structure C (bottom) based on the educt ratio PEI-II/Lac 1 : 5 and 1 : 0.4, respectively.
Figure 8-SI  $^{13}$C NMR spectra of 2-Mal-III based on the substrate ratio PEI-II/Mal-III 1 : 5.
Figure 9-SI. $^{13}$C NMR spectra of (A) 4-Mal-III with structure B based on the substrate ratio PEI-II/Mal-III 1:0.5 (R = reductively coupled maltotriose).
Figure 10-SI  $^{13}$C NMR spectrum of 6-Mal-VII with structure C based on the educt ratio PEI-II/Mal-VII 1 : 0.5 (R = reductively coupled maltoheptaose unit).
Figure 11-SI. ATR-IR spectrum of PEI-II.

Figure 12-SI. ATR-IR spectrum of 2-Glc with structure A.
Figure 13-SI. ATR-IR spectrum of 2-Mal with structure A.

Figure 14-SI. ATR-IR spectrum of 5-Mal-III with structure B.
Figure 15-SI. ATR-IR spectrum of 4-Mal with structure B.

Figure 16-SI. ATR-IR spectrum of 2-Lac with structure A.
**Figure 17-SI.** Binding of ATP with 7-Mal-III (A) Titration of ATP (0.1 mM) to HEPES buffer and (B) to 7-Mal-III in HEPES buffer at 25°C. Graphs show the calorimetric traces (heat flow against time).
Figure 18-SI  -fold increase in nucleotide uptake upon complexation (HepG2 cells): procedure as mentioned for Figure 8.
### Table 1-SI. Influence of the substrate ratio PEI-II : oligosaccharide (OS) and PEI-III : OS on the degree of functionalization (DF), total degree of functionalization (TDF) of modified PEI and the determination of the degree of T, L and D units obtained from elemental analysis.

<table>
<thead>
<tr>
<th>Substrate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PEI</th>
<th>Educt ratio PEI : OS</th>
<th>DF for L + 2xT&lt;sup&gt;b,c&lt;/sup&gt;</th>
<th>TDF for L + 2xT + D&lt;sup&gt;b,d&lt;/sup&gt;</th>
<th>T unit&lt;sup&gt;b&lt;/sup&gt;</th>
<th>L unit&lt;sup&gt;b&lt;/sup&gt;</th>
<th>D unit&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Mal (A)</td>
<td>PEI-III</td>
<td>1 : 4.25</td>
<td>91</td>
<td>70</td>
<td>-</td>
<td>9</td>
<td>91</td>
</tr>
<tr>
<td>5-Mal (B)</td>
<td>PEI-III</td>
<td>1 : 0.5</td>
<td>75</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Mal-III (A)</td>
<td>PEI-III</td>
<td>1 : 4.25</td>
<td>78</td>
<td>60</td>
<td>-</td>
<td>22</td>
<td>78</td>
</tr>
<tr>
<td>5-Mal-III (B)</td>
<td>PEI-III</td>
<td>1 : 2</td>
<td>48</td>
<td>37</td>
<td>-</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>7-Mal-III (C)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>PEI-III</td>
<td>1 : 0.4</td>
<td>30</td>
<td>21</td>
<td>3</td>
<td>67</td>
<td>30</td>
</tr>
<tr>
<td>2-Lac (A)</td>
<td>PEI-II</td>
<td>1 : 5</td>
<td>80</td>
<td>61</td>
<td>-</td>
<td>21</td>
<td>79</td>
</tr>
<tr>
<td>6-Lac (C)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>PEI-II</td>
<td>1 : 0.4</td>
<td>30</td>
<td>23</td>
<td>2</td>
<td>67</td>
<td>31</td>
</tr>
<tr>
<td>3-Lac (A)</td>
<td>PEI-III</td>
<td>1 : 4.25</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5-Lac (B)</td>
<td>PEI-III</td>
<td>1 : 0.6</td>
<td>44</td>
<td>34</td>
<td>-</td>
<td>55</td>
<td>45</td>
</tr>
</tbody>
</table>

<sup>a</sup>Character in brackets presents structure for PEI derivative in Scheme 1. <sup>b</sup>Calculation based on elemental analysis; further details are given in Supporting Information. <sup>c</sup>2xT means that two oligosaccharides can be coupled on one T unit. L means that one oligosaccharide can be coupled on the L unit. <sup>d</sup>All branching units are considered for the calculation of functionalization. <sup>e</sup>Degree of structure units determined by quantitative <sup>13</sup>C NMR. <sup>f</sup>Degree of branching 93 %, using Fréchet equation, based on quantitative <sup>13</sup>C NMR. <sup>g</sup>Degree of branching 94 %, using Fréchet equation, based on quantitative <sup>13</sup>C NMR.
Table 2-SI. Comparison of $^{13}$C chemical shifts of T (-NH$_2$), L (-NHR) and D (-NR$_2$) units for PEI-II and PEI-III and (oligo-)saccharide-modified PEI based on modified PEI-II and PEI-III in D$_2$O.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Structure</th>
<th>D units</th>
<th>L units</th>
<th>T units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D$-$CH_2$-$CH_2$-$T$</td>
<td>$D$-$CH_2$-$CH_2$-$L$</td>
<td>$L$-$CH_2$-$CH_2$-$D$</td>
<td>$L$-$CH_2$-$CH_2$-$L$</td>
</tr>
<tr>
<td>PEI-II</td>
<td>-</td>
<td>58.7</td>
<td>55.6, 56.7</td>
<td>53.5, 54.4</td>
</tr>
<tr>
<td>2-Glc</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>53.3</td>
</tr>
<tr>
<td>2-Mal</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>53.9</td>
</tr>
<tr>
<td>4-Mal</td>
<td>B</td>
<td>-</td>
<td>54.7</td>
<td>53.9</td>
</tr>
<tr>
<td>6-Mal</td>
<td>C</td>
<td>$^a,b$</td>
<td>55.1</td>
<td>53.7</td>
</tr>
<tr>
<td>2-Mal-III</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>54.1</td>
</tr>
<tr>
<td>4-Mal-III</td>
<td>B</td>
<td>-</td>
<td>54.9</td>
<td>53.9</td>
</tr>
<tr>
<td>6-Mal-III</td>
<td>C</td>
<td>$^a,b$</td>
<td>55.3</td>
<td>54.0</td>
</tr>
<tr>
<td>6-Mal-VII</td>
<td>C</td>
<td>58.4</td>
<td>55.2</td>
<td>54.0</td>
</tr>
<tr>
<td>2-Lac</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>54.0</td>
</tr>
<tr>
<td>6-Lac</td>
<td>C</td>
<td>$^a,b$</td>
<td>54.6</td>
<td>53.7</td>
</tr>
</tbody>
</table>

| PEI-III   | -         | 58.8, 58.9 | 55.8 | 53.7, 54.6 | 53.3, 53.7 | 50.4, 50.5 | 48.3 | 42.6 | 40.5 |
| 3-Mal     | A         | -         | -      | 53.8      | -          | -        | 48.1 | -    | -    |
| 5-Mal-III | B         | -         | 54.9    | 53.9      | $^b$      | 49.7 | 48.4 | -    | -    |
| 7-Mal-III | C         | $^a,b$   | 54.9    | 53.9      | $^b$      | 49.7 | 48.4 | 41.6 | 40.1 |

$^a$ Not observable or not detectable compared to unmodified PEI-II. $^b$ Overlapped by other branching units $D$-$CH_2$-$CH_2$-$L$ and $D$-$CH_2$-$CH_2$-$D$. 
Table 3-SI. $^{13}$C signal assignment for PEI-bonded glucose (Glc), maltose (Mal) and maltotriose (Mal-III)$^{ab}$

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reductive Unit 1</th>
<th>Reductive Unit 2</th>
<th>Reductive Unit 3</th>
<th>Reductive Unit 4</th>
<th>Reductive Unit 5</th>
<th>Reductive Unit 6</th>
<th>Reductive Unit 7</th>
<th>Reductive Unit 8</th>
<th>Reductive Unit 9</th>
<th>Reductive Unit 10</th>
<th>Reductive Unit 11</th>
</tr>
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<tbody>
<tr>
<td>Glc</td>
<td>59.4</td>
<td>71.6</td>
<td>78.2</td>
<td>76.7</td>
<td>73.7</td>
<td>65.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mal</td>
<td>60.3</td>
<td>71.4</td>
<td>74.5</td>
<td>85.4</td>
<td>75.5</td>
<td>65.3</td>
<td>103.6</td>
<td>74.6</td>
<td>75.9</td>
<td>72.3</td>
<td>75.6</td>
</tr>
<tr>
<td>Mal-III</td>
<td>60.3</td>
<td>71.5</td>
<td>74.5</td>
<td>85.4</td>
<td>75.6</td>
<td>65.3</td>
<td>103.4</td>
<td>74.4</td>
<td>76.4</td>
<td>79.8</td>
<td>74.0</td>
</tr>
</tbody>
</table>

$^{a}$ Solvent: D$_2$O; reference: internal sodium salt of 3-(trimethylsilyl)propionic acid-2,2,3,3-d$_4$ ($\delta^{13}$C = 0 ppm). $^{b}$ For atom number compare Figures 3 and 5-SI. For signal groups or broadened signals the given $\delta^{13}$C value is the center. $^{c}$ Reductive unit is connected to the PEI scaffold by secondary or primary amino surface groups of PEI.