Hyperbranched PEI with Various Oligosaccharide Architectures

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Published in:
Biomacromolecules

DOI:
10.1021/bm801310d

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Download date: 31-07-2024
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

\[ M_{\text{Polymer}} = (C_2H_5N) \times 84 + a \times (C_{12}H_{23}O_{10}) \]

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

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:

DP = 84, M_n = 3600 g/mol, a = number of coupled maltose

\[ M_{\text{Polymer}} = (C_2H_5N) \times 84 + a \times (C_{12}H_{23}O_{10}) \]

Nitrogen content: \[ N = \frac{84 \times 14}{M_{\text{Polymer}}} \]
Carbon content: \[ C = \frac{(2 \times 12 \times 84 + a \times 12 \times 12)}{M_{\text{Polymer}}} \]
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 13C 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 D2O.

Table 3-SI  13C 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>Substratea</th>
<th>PEI</th>
<th>Educt ratio PEI : OS</th>
<th>DF for L + 2xTb,c</th>
<th>TDF for L + 2xT + Db,d</th>
<th>T unitb</th>
<th>L unitb</th>
<th>D unitb</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>-e</td>
<td>-e</td>
<td>-e</td>
<td>-e</td>
<td>-e</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)</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>7-Mal-III (C)</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)</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>

a Character in brackets presents structure for PEI derivative in Scheme 1. b Calculation based on elemental analysis; further details are given in Supporting Information. e 2xT means that two oligosaccharides can be coupled on one T unit. L means that one oligosaccharide can be coupled on the L unit. d All branching units are considered for the calculation of functionalization. e Degree of structure units determined by quantitative 13C NMR. f Degree of branching 93 %, using Fréchet equation, based on quantitative 13C NMR. g Degree of branching 94 %, using Fréchet equation, based on quantitative 13C 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>$D$-$CH_2$-$CH_2$-$D$</td>
<td>$L$-$CH_2$-$CH_2$-$T$</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-IV</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>
<tr>
<td>PEI-III</td>
<td>-</td>
<td>58.8, 58.9</td>
<td>55.8</td>
<td>53.7, 54.6</td>
</tr>
<tr>
<td>3-Mal</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>53.8</td>
</tr>
<tr>
<td>5-Mal-III</td>
<td>B</td>
<td>-</td>
<td>54.9</td>
<td>53.9</td>
</tr>
<tr>
<td>7-Mal-III</td>
<td>C</td>
<td>-$^{a,b}$</td>
<td>54.9</td>
<td>53.9</td>
</tr>
</tbody>
</table>

$^a$ 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)$^{a,b}$

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reductive Unit</th>
<th>Terminal Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glc</strong></td>
<td>59.4 71.6 78.2 76.7 73.7 65.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Reductive Unit I</strong></td>
<td><strong>Terminal Unit II</strong></td>
</tr>
<tr>
<td>Mal</td>
<td>60.3 71.4 74.5 85.4 75.5 65.3 103.6 74.6 75.9 72.3 75.6 63.4</td>
<td></td>
</tr>
<tr>
<td>Mal-III</td>
<td>60.3 71.5 74.5 85.4 75.6 65.3 103.4 74.4 76.4 79.8 74.0 63.4 102.7 74.7 75.9 72.3 75.7 63.5</td>
<td></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.