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Published in:
Biomacromolecules

DOI:
[10.1021/bm801310d](https://doi.org/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):

Appelhans, D., Komber, H., Quadir, M. A., Richter, S., Schwarz, S., van der Vlist, J., Aigner, A., Mueller, M., Loos, K., Seidel, J., Arndt, K-F., Haag, R., Voit, B., Müller, M., & Seidel, J. (2009). Hyperbranched PEI with Various Oligosaccharide Architectures: Synthesis, Characterization, ATP Complexation, and Cellular Uptake Properties. *Biomacromolecules*, 10(5), 1114-1124. <https://doi.org/10.1021/bm801310d>

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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

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

Nitrogen content: $N = 84 \times 14 / M_{Polymer}$

Carbon content: $C = (2 \times 12 \times 84 + a \times 12 \times 12) / M_{Polymer}$

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:
 $77.7 / (2 \times 27.4 + 30.8) = 91 \%$

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): $77.7 / (2 \times 27.4 + 30.8 + 25.8) = 70 \%$

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 (Σ 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-I** 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

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

Nitrogen content: $N = 84 \times 14 / M_{Polymer}$

Carbon content: $C = (2 \times 12 \times 84 + a \times 12 \times 12) / M_{Polymer}$

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

Figure 3-SI ^1H 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 ^1H 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_2O .

Table 3-SI ^{13}C signal assignment for PEI-bonded glucose (**Glc**), maltose (**Mal**) and maltotriose (**Mal-III**)

Figure 1-SI ^1H 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 ^1H NMR spectrum of **2-Lac** with structure **A** obtained from educt ratio **PEI-II/Lac** 1 : 5.

Figure 3-SI ^1H 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 ^1H 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.

Substrate ^a	PEI	Educt ratio PEI : OS	DF for L + 2xT ^{b,c} %	TDF for L + 2xT + D ^{b,d} %	T unit ^b %	L unit ^b %	D unit ^b %
3-Mal (A)	PEI-III	1 : 4.25	91	70	-	9	91
5-Mal (B)	PEI-III	1 : 0.5	- ^e	- ^e	- ^e	- ^e	- ^e
3-Mal-III (A)	PEI-III	1 : 4.25	78	60	-	22	78
5-Mal-III (B)	PEI-III	1 : 2	48	37	-	51	49
7-Mal-III (C)^f	PEI-III	1 : 0.4	30	21	3	67	30
					7 ^e	53 ^e	40.0 ^e
2-Lac (A)	PEI-II	1 : 5	80	61	-	21	79
6-Lac (C)^g	PEI-II	1 : 0.4	30	23	2	67	31
					6 ^e	52 ^e	42 ^e
3-Lac (A)	PEI-III	1 : 4.25	50	-	-	-	-
5-Lac (B)	PEI-III	1 : 0.6	44	34	-	55	45

^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. ^c 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 (-NH₂), L (-NHR) and D (-NR₂) units for **PEI-II** and **PEI-III** and (oligo-)saccharide-modified PEI based on modified **PEI-II** and **PEI-III** in D₂O.

Substrate		D units			L units		T units		
	Structure	<i>D</i> -CH ₂ - CH ₂ - <i>T</i>	<i>D</i> -CH ₂ - CH ₂ - <i>L</i>	<i>D</i> -CH ₂ - CH ₂ - <i>D</i>	<i>L</i> -CH ₂ - CH ₂ - <i>T</i>	<i>L</i> -CH ₂ - CH ₂ - <i>L</i>	<i>D</i> -CH ₂ - CH ₂ - <i>L</i>	<i>L</i> -CH ₂ - CH ₂ - <i>T</i>	<i>D</i> -CH ₂ - CH ₂ - <i>T</i>
PEI-II	-	58.7	55.6, 56.7	53.5, 54.4	53.2, 53.3	50.2	48.1	42.4	40.33
2-Glc	A	-	-	53.3	-	-	47.8	-	-
2-Mal	A	-	-	53.9	-	-	48.2	-	-
4-Mal	B	-	54.7	53.9	^{-b}	49.4	48.3	-	-
6-Mal	C	^{-a,b}	55.1	53.7	^{-b}	49.6	48.2	41.5	40.0
2-Mal-III	A	-	-	54.1	-	48.3	48.3	-	-
4-Mal-III	B	-	54.9	53.9	^{-b}	50.0	48.4	-	-
6-Mal-III	C	^{-a,b}	55.3	54.0	^{-b}	49.9	48.4	41.7	40.3
6-Mal-VII	C	58.4	55.2	54.0	54.0	49.4	48.4	41.8	40.3
2-Lac	A	-	-	54.0	-	-	48.2	-	-
6-Lac	C	^{-a,b}	54.6	53.7	^{-b}	49.5	48.2	41.5	40.0
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*-CH₂-CH₂-*L* and *D*-CH₂-CH₂-*D*.

Table 3-SI. ^{13}C signal assignment for PEI-bonded glucose (**Glc**), maltose (**Mal**) and maltotriose (**Mal-III**)^{a,b}

substrate	reductive unit ^c																		
	1	2	3	4	5	6													
Glc	59.4	71.6	78.2	76.7	73.7	65.7													
	reductive unit I ^c						terminal unit II												
Mal	60.3	71.4	74.5	85.4	75.5	65.3	103.6	74.6	75.9	72.3	75.6	63.4							
	reductive unit I ^c						middle unit II						terminal unit III						
Mal-III	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	

^a Solvent: D₂O; reference: internal sodium salt of 3-(trimethylsilyl)propionic acid-2,2,3,3-d₄ ($\delta(^{13}\text{C}) = 0$ ppm). ^b For atom number compare Figures 3 and 5-SI. For signal groups or broadened signals the given $\delta(^{13}\text{C})$ value is the center. ^c reductive unit is connected to the PEI scaffold by secondary or primary amino surface groups of PEI.