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Hyperbranched PEI with Various Oligosaccharide Architectures

Appelhans, Dietmar; Komber, Hartmut; Quadir, Mohiuddin Abdul; Richter, Sven; Schwarz, Simona; van der Vlist, Jereon; Aigner, Achim; Mueller, Martin; Loos, Katja; Seidel, Juergen

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Supporting Information

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

Dietmar Appelhans,^{a,} Hartmut Komber,^a Mohiuddin Abdul Quadir,^b Sven Richter,^b Achim Aigner,^c Katja Loos,^d Martin Müller,^a Jürgen Seidel,^e Karl-Friedrich Arndt,^f Rainer Haag,^{b,*} Brigitte Voit^a*

^a Leibniz Institute of Polymer Research Dresden, Hohe Strasse 6, D-01069 Dresden, Germany

^b Institut für Chemie und Biochemie, Freie Universität Berlin, Takustr. 3, D-14195 Berlin, Germany

^c Department of Pharmacology and Toxicology, Philipps-University Marburg, School of Medicine, Karl-v.-Frisch-Strasse 1, D-35033 Marburg, Germany

^d Faculty of Mathematics and Natural Sciences, Laboratory of Polymer Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

^e Institute of Physical Chemistry, TU Bergakademie Freiberg, Leipziger Str. 29, D-09596 Freiberg, Germany

^f Physical Chemistry of Polymers, Department of Chemistry, TU Dresden, Mommenstr. 4, D-01069 Dresden, Germany

applhans@ipfdd.de and haag@chemie.fu-berlin.de

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 ($\Sigma 58.2$) 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-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**)

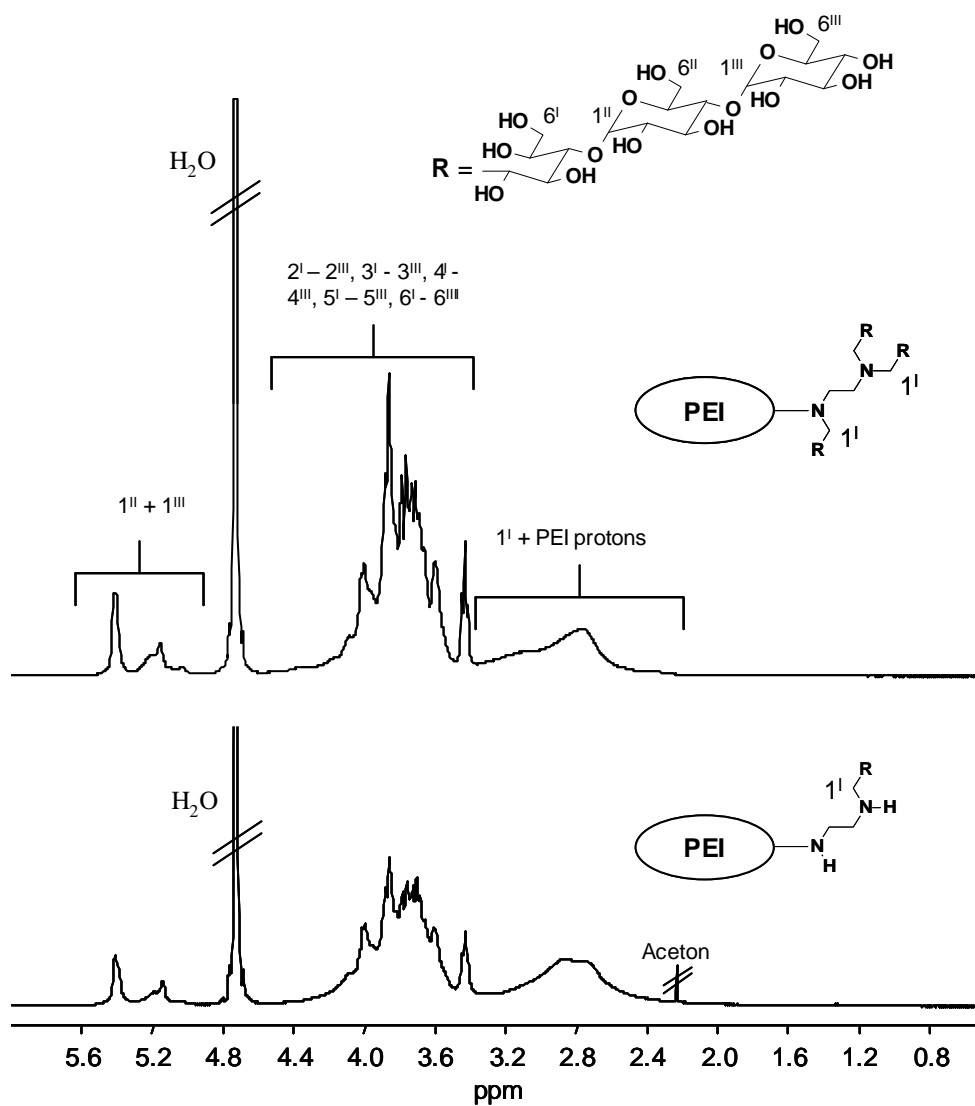


Figure 1-SI ¹H spectra of **1-Mal-III** with structure **A** (top) and **3-Mal-III** with structure **B** (bottom) obtained from educt ratio **PEI-I/Mal-III** 1 : 5 and 1 : 2, respectively.

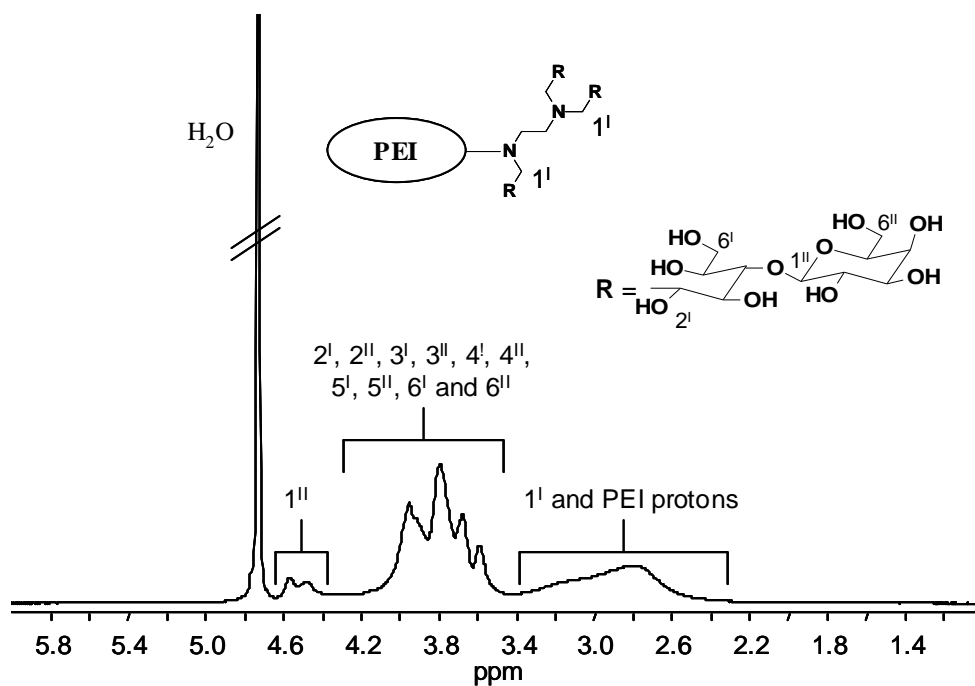


Figure 2-SI ^1H NMR spectrum of 2-Lac with structure A obtained from educt ratio PEI-II/Lac 1 : 5.

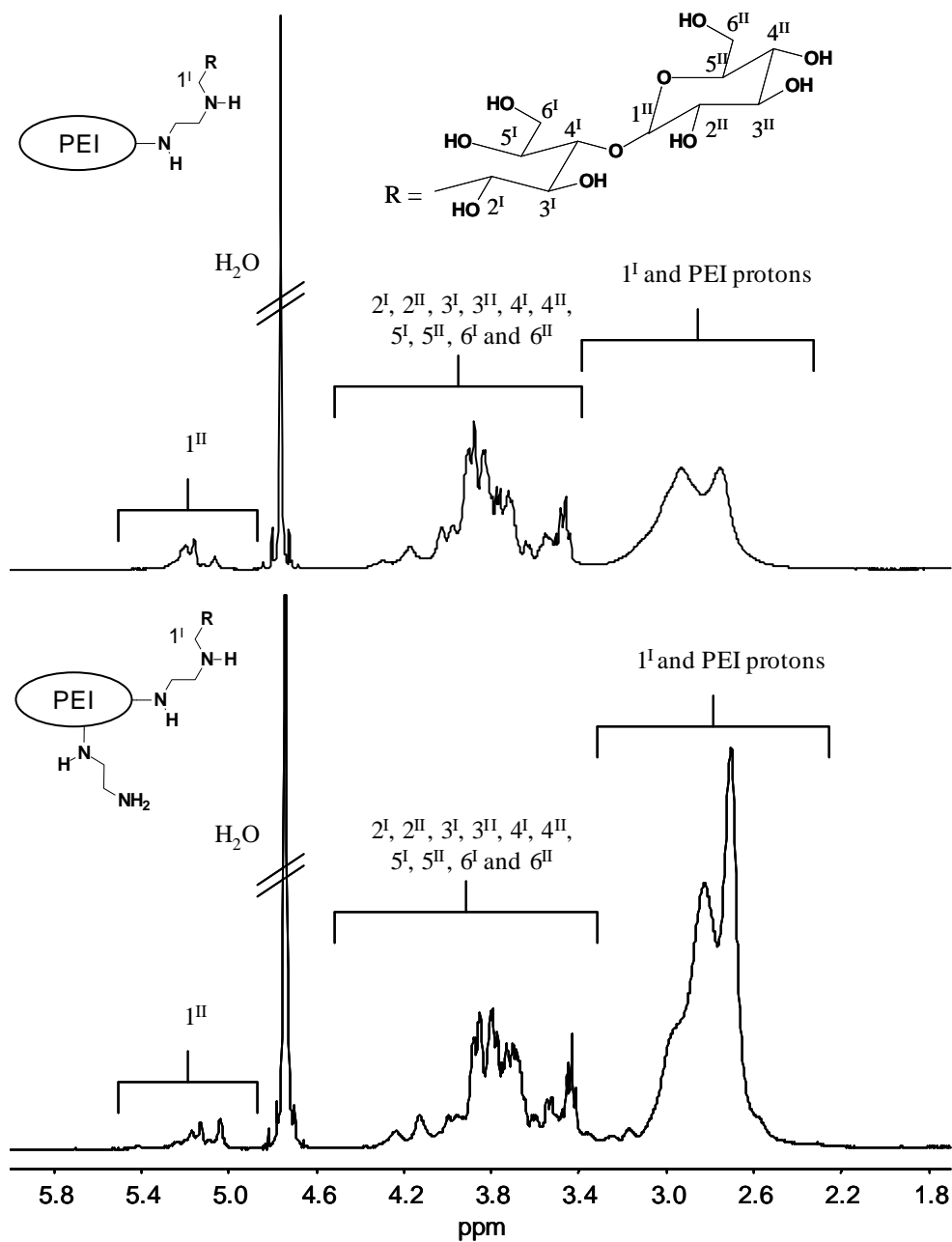


Figure 3-SI ^1H spectra of **4-Mal** with structure **B** (top) and **6-Mal** with structure **C** (bottom) obtained from educt ratio **PEI-II/Mal** 1 : 0.5 and 1 : 0.2, respectively.

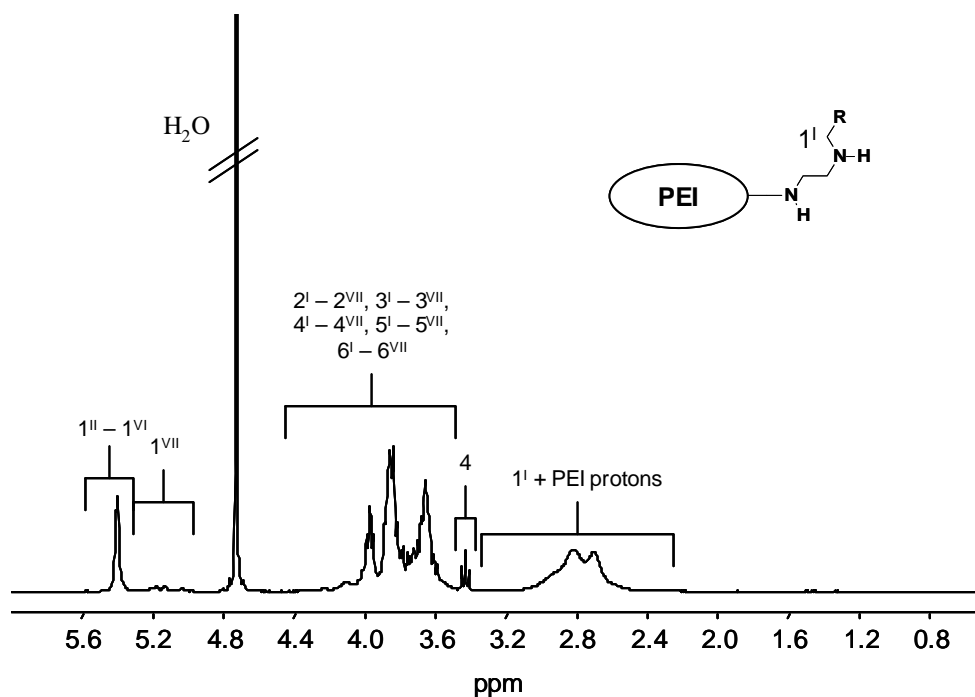


Figure 4-SI ^1H NMR spectrum of **6-Mal-VII** with structure **C** based on the educt ratio **PEI-II/Mal-VII** 1 : 0.5 (R = reductively coupled maltoheptaose unit; Scheme 1).

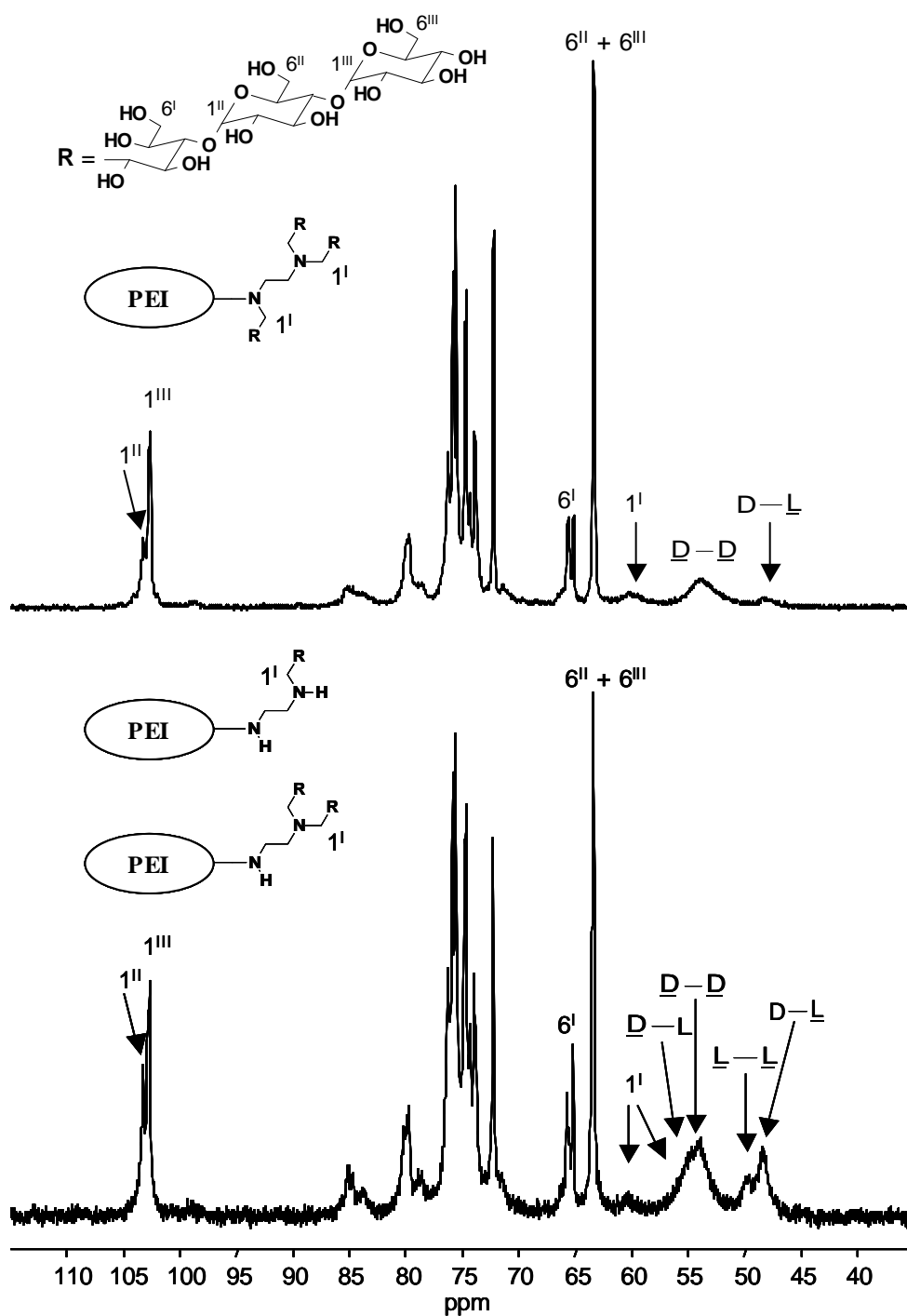


Figure 5-SI ¹³C NMR spectra of **1-Mal-III** with structure **A** (top) based on the educt ratio **PEI-I/Mal-III** 1 : 5 and **3-Mal-III** with transition from structure **A** to **B** (bottom) based on the educt ratio **PEI-III/Mal-III** 1 : 2.

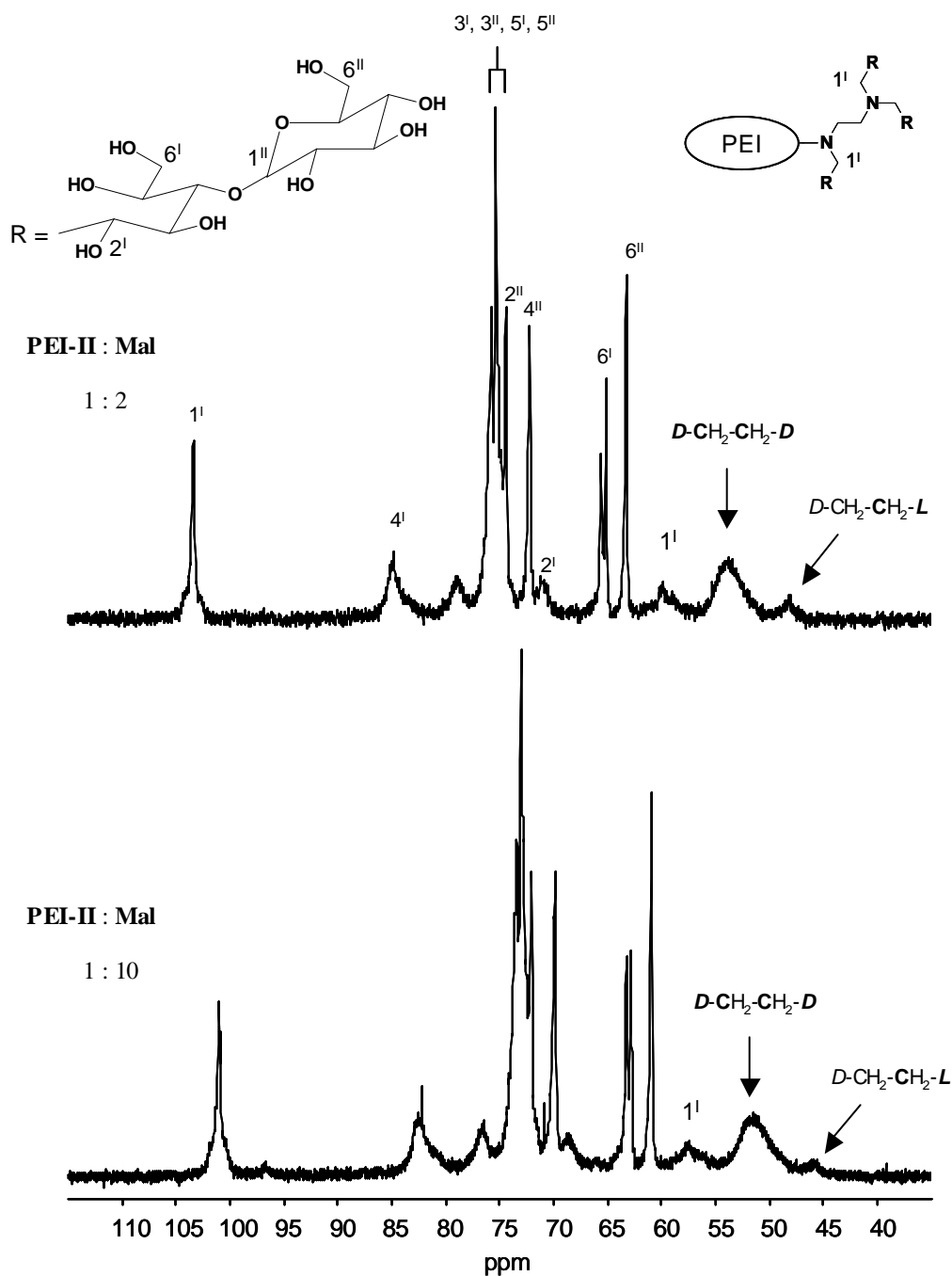


Figure 6-SI ^{13}C NMR spectra of 2-Mal obtained from educt ratio PEI-II/Mal 1 : 2 and 1 : 10, respectively.

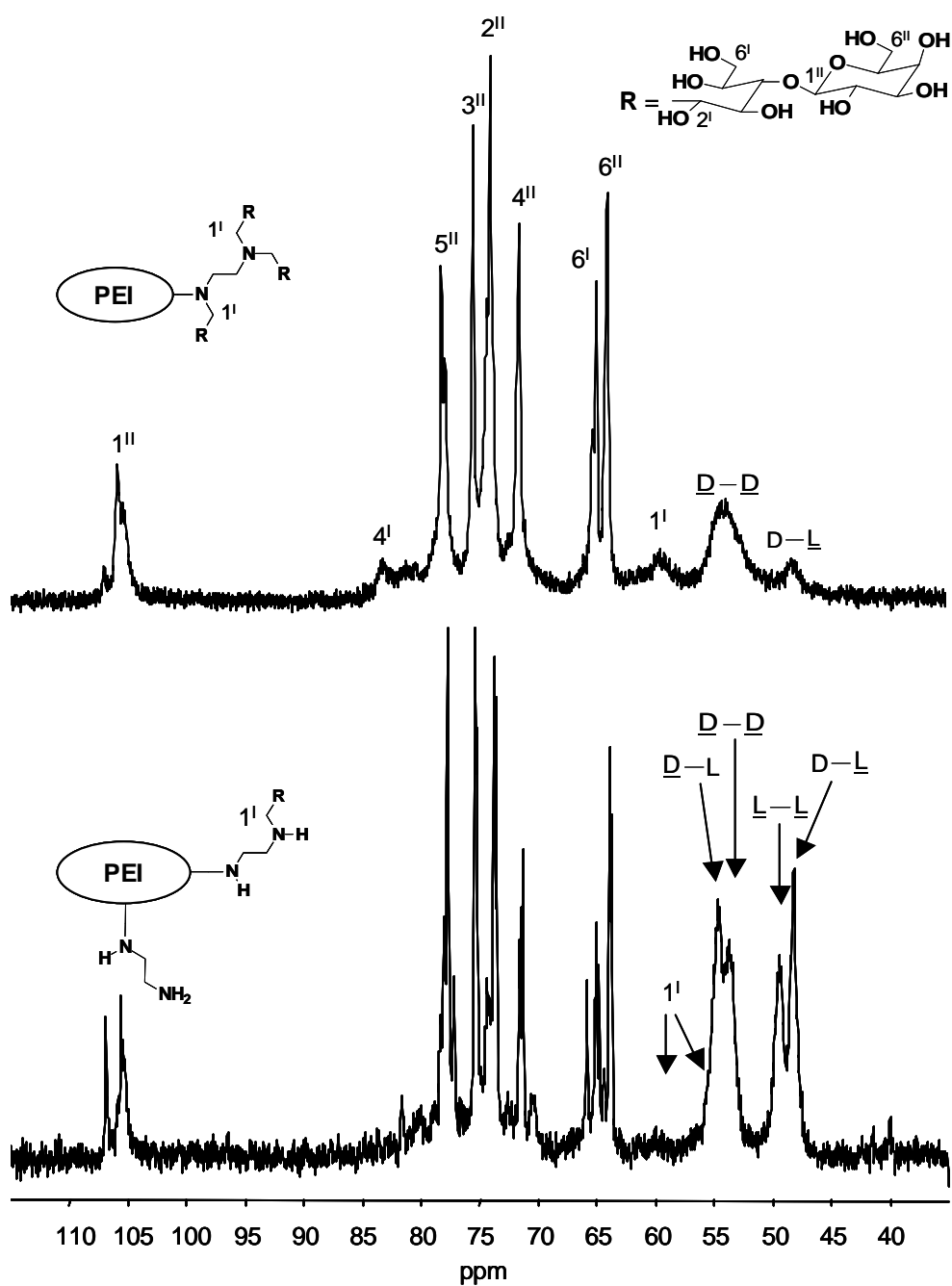


Figure 7-SI ^{13}C NMR spectrum of **2-Lac** with structure **A** (top) and **6-Lac** with structure **C** (bottom) based on the educt ratio **PEI-II/Lac** 1 : 5 and 1 : 0.4, respectively.

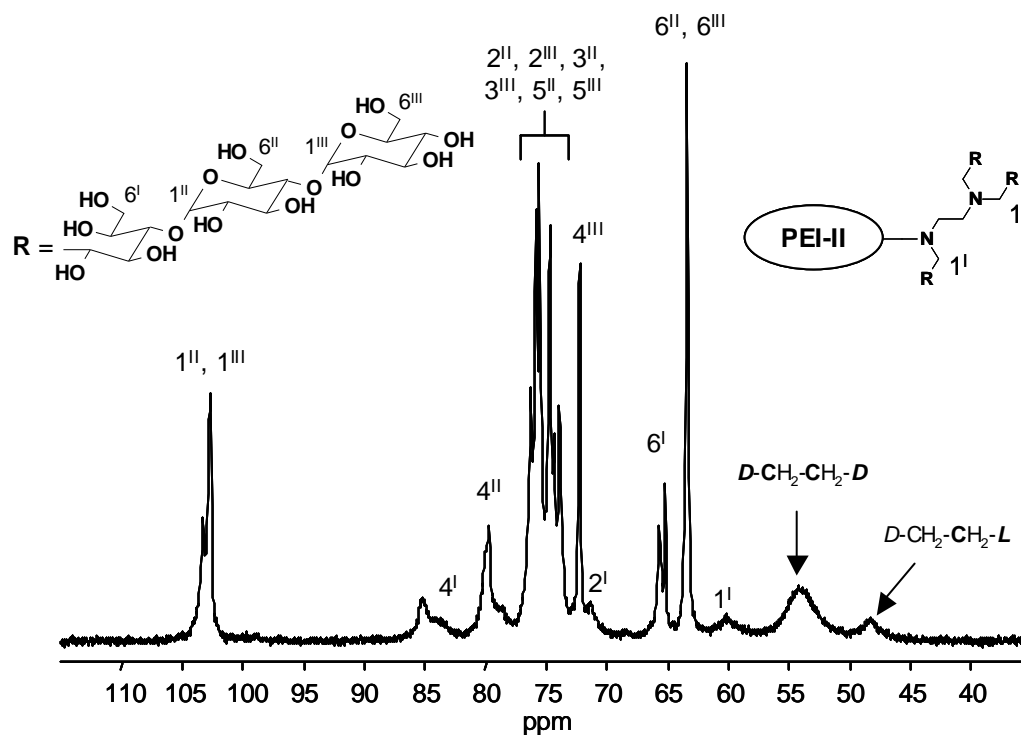


Figure 8-SI ^{13}C NMR spectra of 2-Mal-III based on the substrate ratio PEI-II/Mal-III 1 : 5.

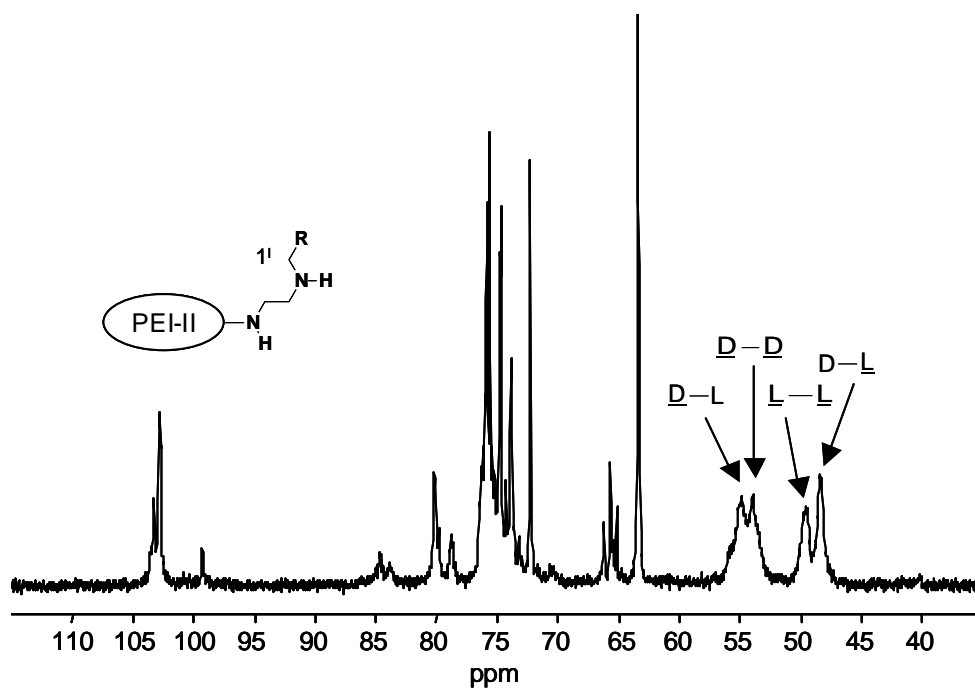


Figure 9-SI. ^{13}C NMR spectra of (A) **4-Mal-III** with structure **B** based on the substrate ratio **PEI-II/Mal-III** 1 : 0.5 (R = reductively coupled maltotriose).

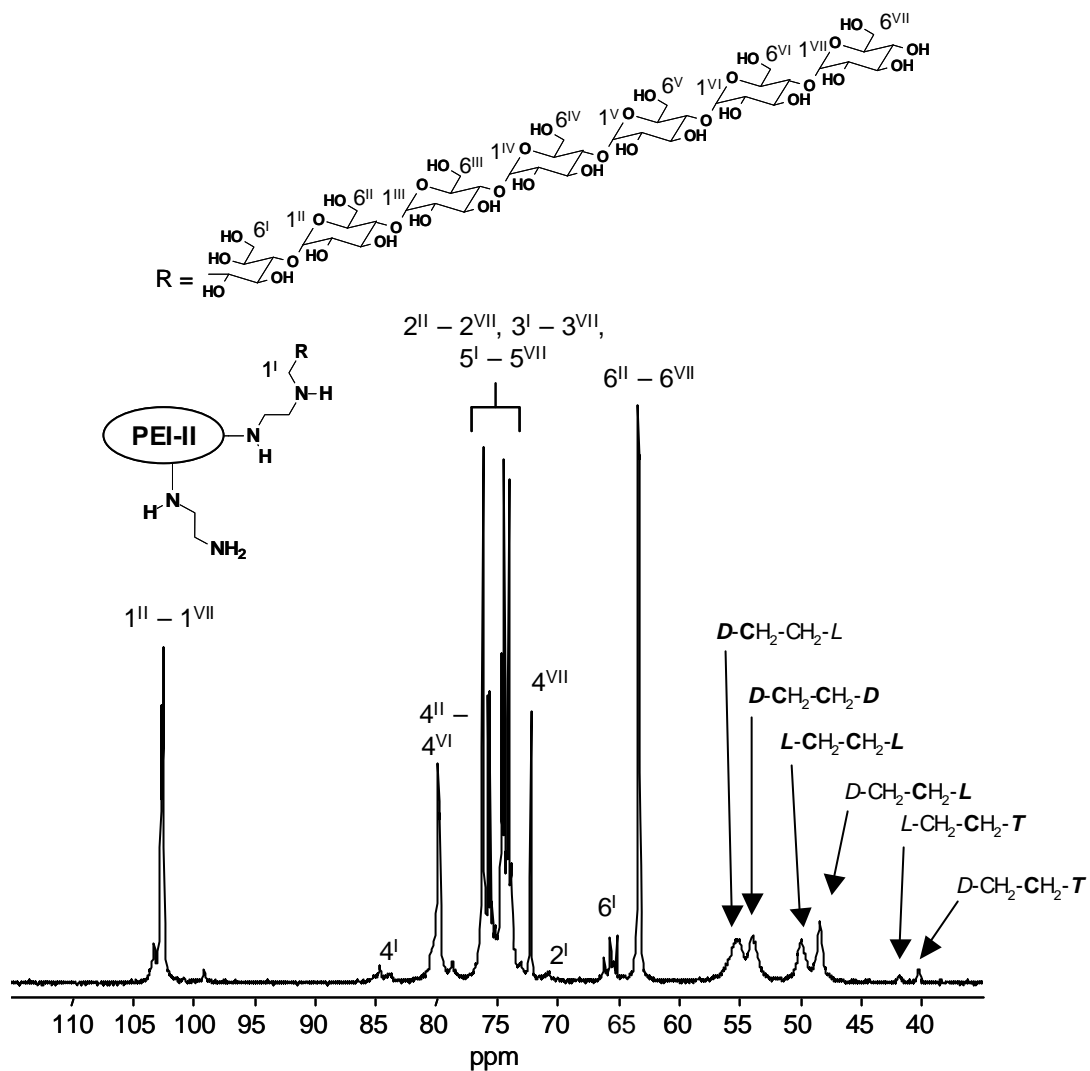


Figure 10-SI ^{13}C NMR spectrum of **6-Mal-VII** with structure **C** based on the educt ratio **PEI-II/Mal-VII** 1 : 0.5 (R = reductively coupled maltoheptaose unit).

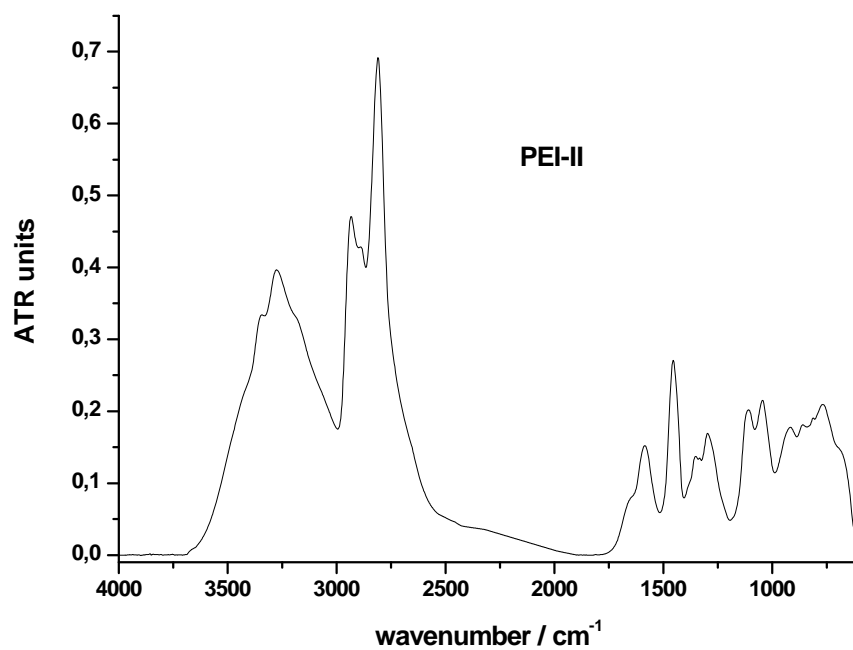


Figure 11-SI. ATR-IR spectrum of **PEI-II**.

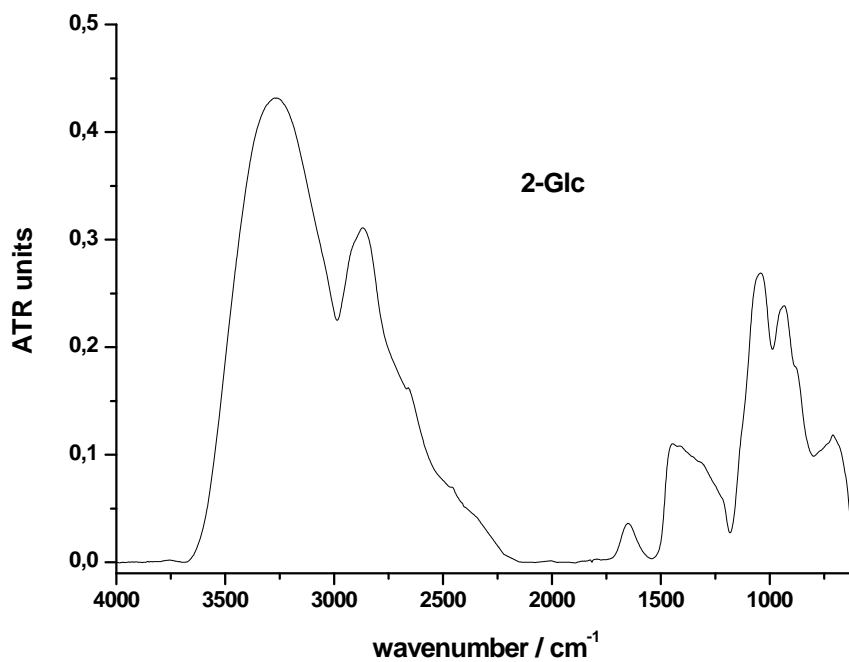


Figure 12-SI. ATR-IR spectrum of **2-Glc** with structure **A**.

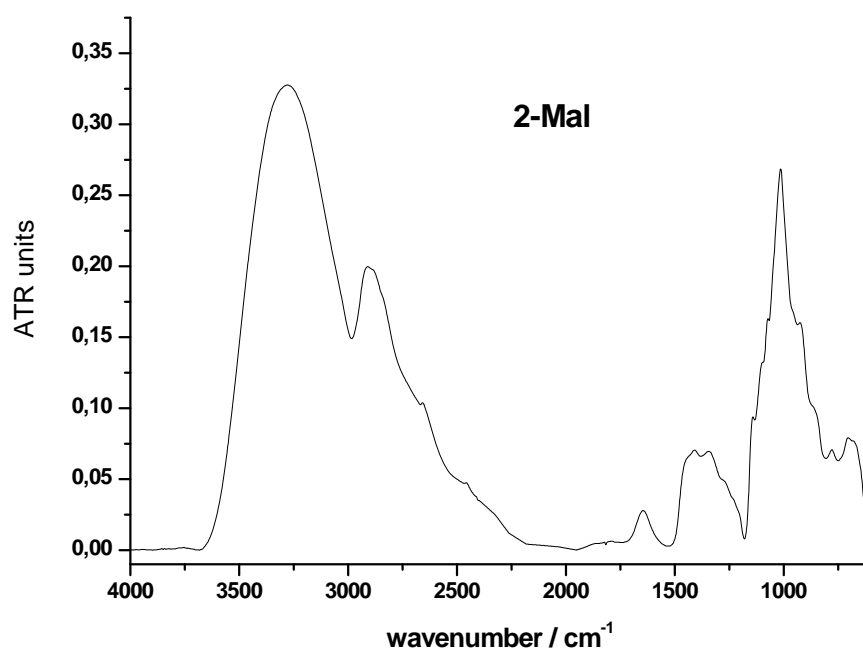


Figure 13-SI. ATR-IR spectrum of **2-Mal** with structure **A**.

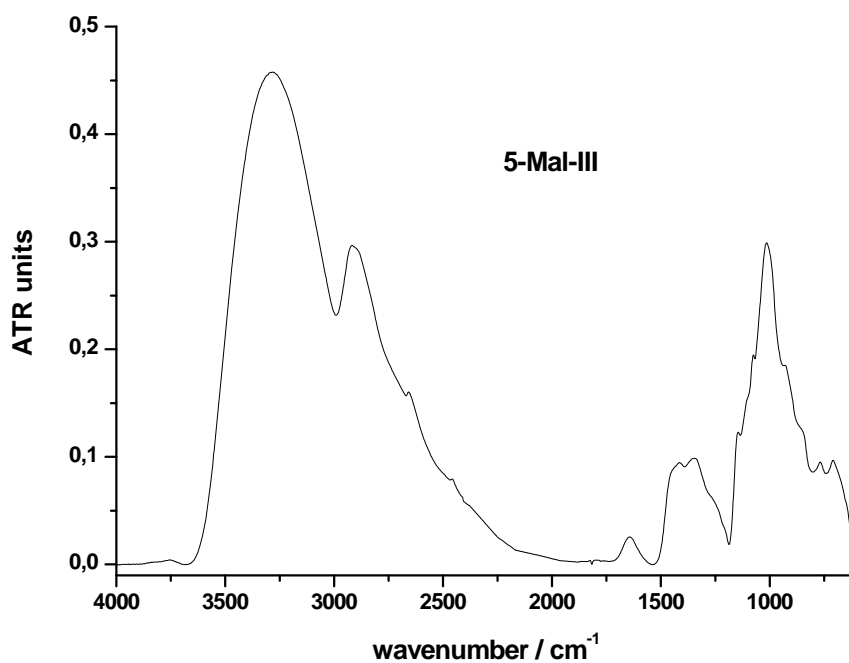


Figure 14-SI. ATR-IR spectrum of **5-Mal-III** with structure **B**.

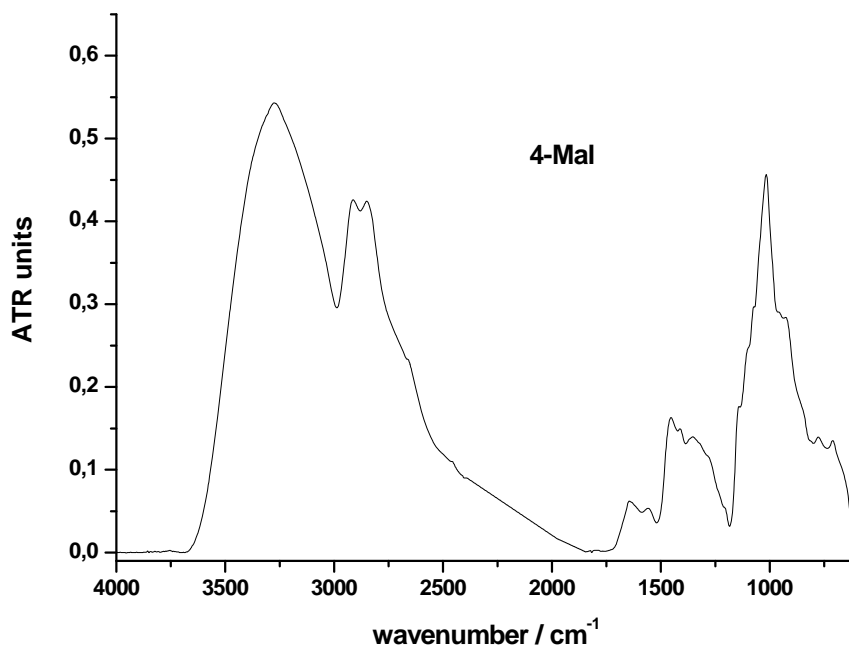


Figure 15.-SI ATR-IR spectrum of **4-Mal** with structure **B**.

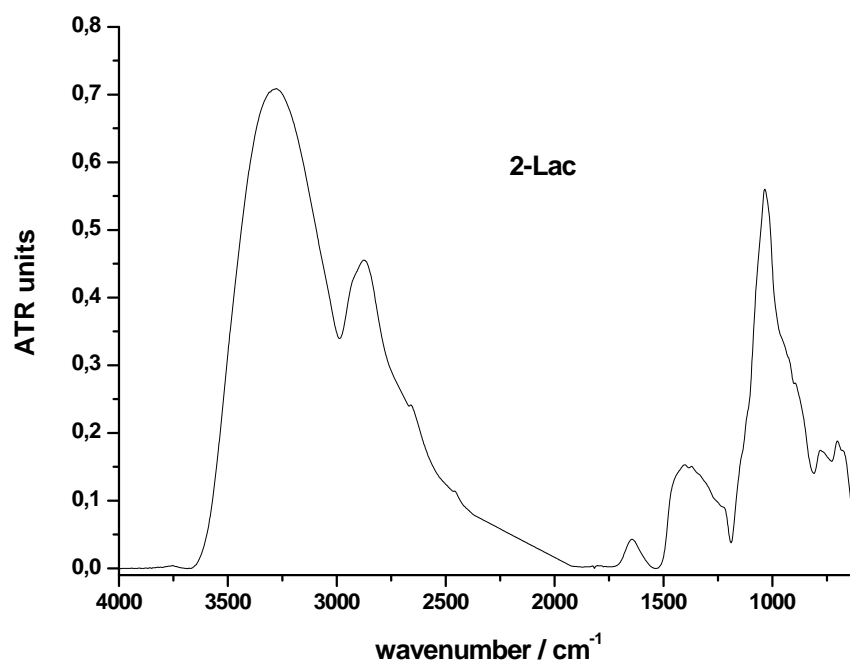


Figure 16-SI. ATR-IR spectrum of **2-Lac** with structure **A**.

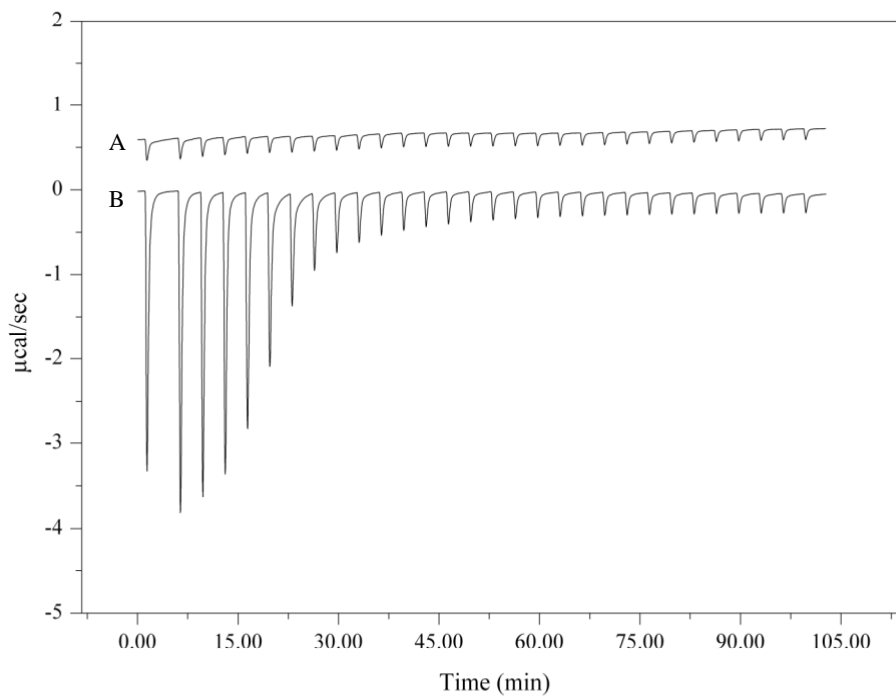


Figure 17-SI. Binding of ATP with **7-Mal-III** (A) Titration of ATP (0.1 mM) to HEPES buffer and (B) to **7-Mal-III** in HEPES buffer at 25°C. Graphs show the calorimetric traces (heat flow against time).

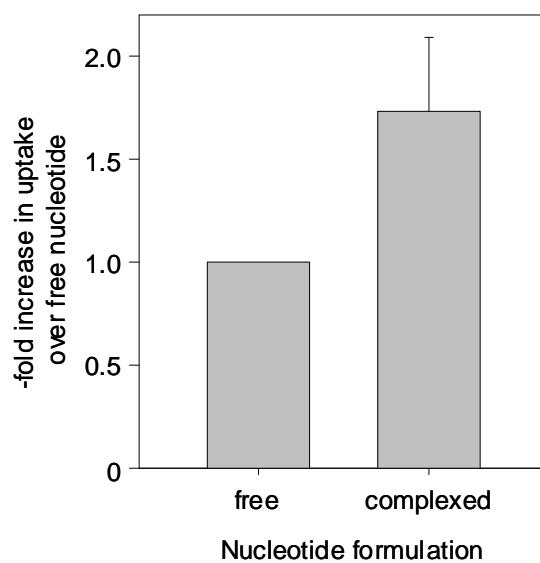


Figure 18-SI -fold increase in nucleotide uptake upon complexation (HepG2 cells): procedure as mentioned for Figure 8.

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 | Structure | D units | | | L units | | | T units | |
|------------------|-----------|---|---|---|---|---|---|---|---|
| | | <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.