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Cyclodextrin-Adamantane Host–Guest Interactions on the Surface of Biocompatible Adamantyl-Modified Glycodendrimers

Marco Paolino,[†,‡,*] Franka Ennen,[‡,§] Stefania Lamponi,[†] Mihaela Cernescu,[⊥] Brigitte Voit,[‡,§] Andrea Cappelli,[†] Dietmar Appelhans,[‡] and Hartmut Komber[‡,*]

[†]Dipartimento di Biotecnologie, Chimica e Farmacia and European Research Centre for Drug Discovery and Development, Università degli Studi di Siena, Via A. Moro, 53100 Siena, Italy

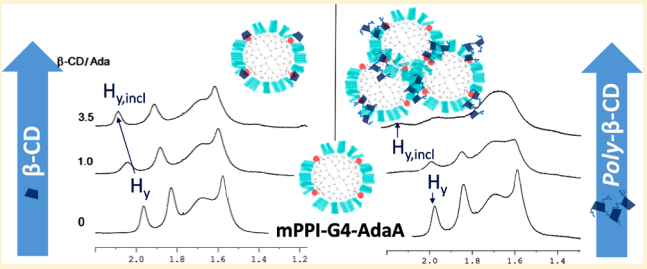
[‡]Leibniz Institute of Polymer Research Dresden, Hohe Strasse 6, 01069 Dresden, Germany

[§]Organic Chemistry of Polymers, Technische Universität Dresden, 01062 Dresden, Germany

[⊥]Institute of Physical and Theoretical Chemistry, Johann-Wolfgang-Goethe University, Max-von-Laue-Strasse 7, 60438 Frankfurt/Main, Germany

Supporting Information

ABSTRACT: A series of adamantyl-modified glycodendrimers (**mPPI-Gx-AdaA-C**) was prepared in a two-step synthesis using two efficient reactions: (1) urea bond formation from amine and isocyanate and (2) reductive amination. ^{1}H NMR spectroscopy (host guest titration and ROESY experiments) was used to evaluate the graded effect of steric hindrance as a function of the number and type of oligosaccharide molecules and of the number of adamantyl (Ada) units on the complexation with monomeric *β*-cyclodextrin (*β*-CD). Glycosylated fourth generation PPIs showing an average substitution in adamantyl groups of 13% were found to interact with *β*-CD effectively, and were considered as candidates for further complexation studies with a polymeric cyclodextrin derivative (*poly-β*-CD). The host–guest interaction features of the maltosylated dense shell glycodendrimer along with the low cytotoxicity provided the rational basis for the use of these adamantyl-functionalized glycodendrimers in the design of supramolecular systems potentially useful as healthcare materials.



1. INTRODUCTION

The control of interactions within supramolecular polymer structures has received growing attention in recent years due to the potential applications of this innovative concept in many fields such as stimuli-responsive gels, nanoparticles, biosensing devices, gene carriers, and more.[1,2] In the building of supramolecular polymer systems, different types of interaction mechanisms have been used such as hydrophobic interactions in polar solvents (e.g., water),[3] electrostatic interactions between polymers with different charge,[4] and molecular recognition mechanisms based on specific interactions such as acceptor donor–hydrogen bonds,[5,6] *π*-stacking interactions,[7−9] or the formation of inclusion complexes as in the case of calixarenes[10] and cyclodextrins.[11]

Cyclodextrins (CDs) are cyclic oligosaccharides formed by monomers of D-glucopyranose bound together by means of *α*-1,4-glucosidic linkages and closed in a ring to form a hollow structure of a truncated cone. Depending on the number of monomers the CDs are classified into *α* (six glucose units), *β* (seven glucose units), or *γ* (eight glucose units). The three-dimensional structure forces the hydroxyl groups on the outer edges, while the internal cavity contains only hydrogen atoms and oxygen bridges. This arrangement ensures that the cyclodextrins possess an external hydrophilic surface and a hydrophobic central cavity.[12−14] The central cavity of cyclodextrins (in particular of the *β*-CD) is extensively used to include hydrophobic molecules in order to increase their solubility in water.[15−17] Moreover, the cyclodextrin structure has been included in a large number of different polymers (including polysaccharides) with the aim of developing advanced drug delivery systems, hydrogel materials, or biological probes.[18−23] Among the many hydrophobic or amphiphilic molecules studied as guests of *β*-CD internal cavity, a special attention has been paid to adamantyl (Ada) moiety because it tightly fits in the *β*-CD cavity resulting in a host–guest inclusion complex with an association constant greater than 10^4 M^{-1}.[24] This interaction was used in various supramolecular systems composed by linear or branched polymers bearing cyclodextrin structure and linear polymers bearing adamantyl groups to build polymeric networks, branched polymers or block copolymers.[25−29] On the contrary, only a few examples are present in literature dealing with the

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use of dendrimers, and in particular glycodendrimers, as basic structures to create supramolecular systems through Ada-β-CD interaction.[30−32]

Dendrimers are a class of macromolecular compounds characterized by a high degree of branching, monodispersity, and structural perfection.[33,34] The large number of reactive groups at their periphery makes the dendrimers an excellent scaffold for building ordered and variously functionalized macromolecules.[35−39] The modification of poly(propyleneimine) (PPI) dendrimers with sugar molecules on the periphery by reductive amination is extensively studied in our research group and these glycosylated PPI dendrimers have been studied for several pharmaceutical and diagnostic applications.[40−42] This structural modification leads to the formation of a glyco-shell on the dendrimer surface that greatly diminishes the toxicity of PPI dendrimers and makes them excellent candidates for the development of drug carriers and diagnostic agents.[43−45]

In this paper, we report on the synthesis of a new class of hybrid PPI dendrimers of fourth (G4) and fifth (G5) generation decorated on their surface with adamantyl moieties (hydrophobic guest groups) and maltose or maltotriose units (biocompatible groups). NMR studies provided evidence about the formation of supramolecular complexes between the hybrid PPI dendrimers and β-CD (host molecules) both in the monomeric form and in the polymeric one (e.g., water-soluble random β-CD-epichlorohydrin polymer). The host−guest interaction behavior of maltosylated dense shell glycodendrimers bearing adamantyl groups was preliminarily characterized by dynamic light scattering (DLS). Finally, the potential cytotoxicity of the component generating complexes was evaluated in view of their use in the development of supramolecular systems for healthcare applications.

2. EXPERIMENTAL SECTION

2.1. Materials. Sodium borate, 1-adamantyl isocyanate, maltose, maltotriose, borane−pyridine complex (BH_3·Pyr), β-cyclodextrin, and chloroform were used as received from Aldrich, Acros, or Fluka; fourth and fifth generation poly(propyleneimine) dendrimers PPI-G4 (M = 3513.86 g/mol) and PPI-G5 (M = 7167.96 g/mol) were used as received from Symo-Chem (Eindhoven, The Netherlands) as DAB-Am-32 and DAB-Am-64, respectively.

β-CD-epichlorohydrin polymer (*poly-β-CD*) was purchased from Sigma-Aldrich (C2485, Lot 118 K1408 V). According to the data sheet, the cyclodextrin content is 50−55 wt % (by iodometric titration of reducing end groups after acid hydrolysis) and the molecular weight distribution =2000−15 000 g/mol with an average molecular weight of 3500 g/mol.

Membrane tubes (ZelluTransRoth VSerie with 1000 MWCO, Carl Roth GmbH&Co, Karlsruhe/Germany) for dialysis were used after washing with deionized water.

Dulbecco's modified Eagle's medium (DMEM) and Eagle's minimum essential medium (EMEM), trypsin solution, and all the solvents used for cell culture were purchased from Lonza (Switzerland). Mouse-immortalized fibroblasts NIH3T3 were purchased from American Type Culture Collection (USA).

2.2. Characterization and Methods. *NMR Spectroscopy.* NMR spectra were recorded on a Bruker Avance III 500 NMR spectrometer operating at 500.13 MHz for ^{1}H and at 125.75 MHz for ^{13}C using a 5 mm quad (^{1}H, ^{13}C, ^{19}F, ^{31}P) inverse probe, equipped with a shielded z-gradient coil. The ^{1}H NMR spectra were recorded applying a spectral width of 6000 Hz digitized into 64K points and a recycle time of 10 s. The solvent signals were used as internal standard: DMSO-d_6: δ(^{1}H) = 2.50 ppm, δ (^{13}C) = 39.6 ppm and $CDCl_3$: δ (^{1}H) = 7.26 ppm, δ (^{13}C) = 77.0 ppm, respectively. Spectra recorded from D_2O solutions were referenced on external sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 (δ (^{1}H) = 0 ppm). In complexation experiments the signal of the anomeric protons of β-CD was set to 4.98 ppm. Temperature was controlled by the Bruker variable temperature accessory BVT-3000 and was calibrated using the standard Wilmad ethylene glycol sample. In all measurements, the temperature was maintained constant at 303 ± 0.2 K.

The concentration of both components in the host guest titration was 1×10^{-3} M in the adamantyl units and β-CD units for the 1:1 complex. However, due to titration of one component by the second one the concentrations are different at different degree of complexation. For the 2D ROESY experiments solutions 8×10^{-3} M in adamantyl units and 1.6×10^{-2} in β-CD units were used (1:2 Ada/β-CD). The 2D ROESY spectrum were recorded with cw spinlock using a loop of 690 ($180°_x$ - $180°_{-x}$) pulses of 180 μs length of the 180° pulse giving a total mixing time of 248 ms (phase sensitive using TPPI, 24 scans, sweep width =4746 Hz, 2K data points in F2 and 256 experiments in F1).

Mass Spectrometry. Laser-induced liquid bead ionization/desorption mass spectrometry (LILBID-MS) is a recently developed method for the soft mass spectrometric investigation of biomolecules and biomolecular complexes.[46] Liquid micro droplets of the aqueous sample fluid (⌀ ∼ 50 μm) are irradiated by synchronized IR laser pulses produced by a Nd/YAG pumped optic parametric oscillator working at 2.9 μm. The energy is transferred into the liquid by resonant excitation of a stretching vibration of the water molecules, which led to the superexcitation and subsequent explosive disruption of the droplet. The molecules thus ejected into vacuum were then analyzed by a TOF mass spectrometer. The dendrimer samples were prepared in aqueous solution with concentrations of 4×10^{-6} M. All measurements were performed in anionic mode.

Dynamic Light Scattering (DLS). To characterize the particle size and size distribution of **mPPI-G4-AdaA** sample and its complexes with β-CD and *poly-β-CD*, dynamic light scattering (DLS) measurements were carried out at 25 °C at a fixed angle of 173° using the Nano Zetasizer (Malvern), equipped with a He−Ne laser (4 mW) and a digital autocorrelator. The particle size distribution was determined using a multimodal peak analysis by intensity, volume and number, respectively.

The DLS measurements were made at concentrations of about 10^{-3} M in adamantyl units and of 2×10^{-3} M in β-CD units in the 1:2 complexes. The investigated solutions were filtered directly through 0.8 mm filters and measured after 24 h.

Determination of β-CD Content and Ratio EP/β-CD in poly-β-CD by ^{1}H NMR Spectroscopy. The ratio between the incorporated epichlorohydrin (EP) and β-CD (EP/β-CD) in *poly-β-CD* was calculated from ^{1}H NMR signal integrals as reported in literature (ref 47) (measurement in D_2O). The signals regions from 4.8 to 5.5 ppm (assigned to the seven anomeric protons) and from 3.0 to 4.5 ppm (assigned to five protons of 2-hydroxypropyl ether units and to the remaining cyclodextrin protons) were considered. The calculated value for the *poly-β-CD* used in this work is EP/β-CD = 6.8 (∼65 wt % β-CD). This value is higher than the value reported in the data sheet (50−55 wt % determined by iodometric titration). Thus, the cyclodextrin content was additionally determined using an exact amount of resorcinol as a standard in a solution of 20.1 mg/mL of *poly-β-CD* in D_2O. The comparison of the integral values of the aromatic protons of the resorcinol and the integral value of the anomeric protons of the cyclodextrin also allows to calculate the content of β-CD in the polymer. This method gives 63.5 wt % confirming the value of ∼65 wt % determined from comonomer-specific signal integrals.

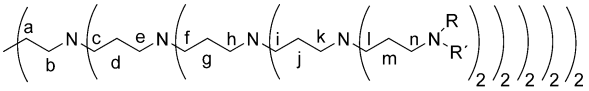
Cell Cultures and Cytotoxicity Assay. NIH3T3 were used for cytotoxicity experiments. NIH3T3 were maintained in DMEM at 37 °C in a humidified atmosphere containing 5% CO_2. The culture medium was supplemented with 10% fetal calf serum (FCS), 1% L-glutamine-penicillin-streptomycin solution, and 1% MEM non essential amino acid solution. Once at confluence, cells were washed with 0.1 M PBS, taken up with trypsin-EDTA solution and then centrifuged at 1000 rpm for 5 min. The pellet was resuspended in medium solution (dilution 1:15), and cells were seeded.

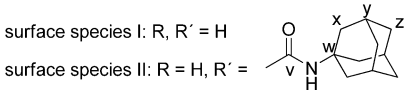
After 24 h of incubation, the following samples, solubilized in physiological solution, were added to the cells: *poly-β*-CD, **PPI-G4-AdaA**, **0.5mPPI-G4-AdaA**, **mPPI-G4-AdaA**, and **m3PPI-G4-AdaA**. The following concentrations for each sample were tested: 1, 5, 10, 50, 100, and 150 μg/mL. Each concentration was tested in triplicate. Cell viability after 24 h of incubation with the different compounds was evaluated by Neutral Red Uptake (Sigma-Aldrich, Switzerland) by the procedure previously reported.[48] Briefly, the following solutions were prepared in order to determine the percentage of viable cells:

1 Neutral Red (NR) stock solution: 0.33 g of NR dye powder in 100 mL of sterile water;
2 NR medium: 1.0 mL of NR stock solution +99.0 mL of routine culture medium prewarmed to 37 °C;
3 NR desorbing solution: 1% glacial acetic acid solution +50% ethanol +49% water (v/v).

At the end of the incubation, the routine culture medium was removed from each well and the cells were carefully rinsed with 1.0 mL of prewarmed Dulbecco's phosphate buffered saline (D-PBS). Multiwells were then gently blotted with paper towels. 1.0 mL of NR medium was added to each well and further incubated at 37 °C, 95% humidity, 5.0% CO_2 for 3 h. The cells were checked during the NR incubation for NR crystal formation. After incubation, the NR medium was removed, and cells were carefully rinsed with 1.0 mL of prewarmed D-PBS. Then, the D-PBS was decanted and blotted from the wells and exactly 1.0 mL of NR desorbing solution was added to each sample. Multiwells were then put on a shaker for 20−45 min to extract NR from the cells and form a homogeneous solution. During this step the samples were covered in order to protect them from light. After 5 min from the plate shaker removal the absorbance was read at 540 nm by a UV/visible spectrophotometer (Lambda 25, Perkin-Elmer).

2.3. Synthesis. *General Procedure for the Synthesis of Partially Adamantyl-Modified Dendrimers.* The appropriate dendrimer (PPI-G4 or PPI-G5) was dissolved in 20 mL of chloroform and the resulting solution was degassed in argon atmosphere for 1 h. The suitable amount of 1-adamantyl isocyanate (ca. 12.5%, 25%, 50% in primary ammine unit) was then added and the resulting mixture was stirred at room temperature for 24 h in argon atmosphere. The solvent was removed under reduced pressure to obtain the expected material.

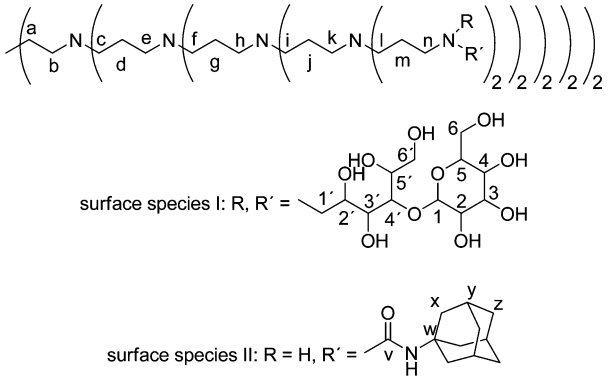




PPI-G4-AdaA. **PPI-G4-AdaA** was prepared from PPI-G4 (0.20 g, 0.057 mmol) and 1-adamantyl isocyanate (0.040 g, 0.23 mmol) and obtained as a colorless oil (0.23 g, yield 94%). The number of adamantyl groups in **PPI-G4-AdaA** was calculated from the ^{1}H NMR spectrum recorded in $CDCl_3$. The intensities of the signals at 2.72 ppm (n in I, unreacted amino groups) and at 3.11 ppm (n in II, reacted amino groups) were evaluated taking into account 32 terminal groups. A value of 4.2 for the adamantyl groups was determined (13% conversion of amino groups) in good agreement with the intended conversion (12.5%). ^{1}H NMR (500 MHz, $CDCl_3$): 1.36 (a), 1.5−1.7 (d, g, j, m), 1.65 (z), 1.96 (x), 2.03 (y), 2.3−2.55 (b, c, e, f, h, i, k, l), 2.72 (n in I), 3.11 (n in II), 5.0−5.6 (br, NH bonded to Ada), 5.7−6.3 ppm (two broad overlapping signals at 5.9 and 6.15 ppm; NH bonded to PPI-G4). ^{13}C NMR (125 MHz, $CDCl_3$): 24−25 (d, g, j), 25.1 (a), 27.5 (m in II), 29.5 (y), 30−31 (m in I), 36.5 (z), 38.1 (n in II), 40.6 (n in I), 42.5 (x), 50.3 (w), 51−53 (c, e, f, h, i, k, l), 54.8 (b), 158.3 ppm (v). ^{1}H NMR (500 MHz, D_2O): 1.35 (a), 1.4−1.75 (d, g, j, m, z), 1.87 (x), 1.97 (y), 2.2−2.5 (b, c, e, f, h, i, k, l), 2.56 (n in I), 2.97 ppm (n in II) (Figure SI-1, Supporting Information). ^{13}C NMR (125 MHz, D_2O): 24.9 (d, g, j), 26.7 (a), 28.0 (n. i.), 29.0 (m in II), 30.8 (m in I), 32.4 (y), 39.4 (z), 40.6 (n in II), 41.9 (n in I), 45.0 (x), 49.9 (n. i.), 52.9 (w), 53−55.5 (c, e, f, h, i, k, l), 56.5 (b), 161.8 ppm (v) (Figure SI-2, Supporting Information). IR: 3285 (NH_2), 2906, 2850, 2799 (CH, CH_2), 1651 (C═O), 1555 cm^{-1} (N−H, NH_2). MALDI−TOF MS: m/z calculated for $C_{228}H_{492}N_{66}O_4$ = 4222.79; found (main peak, Figure SI-6, Supporting Information) 4226.11 (M).

General Procedure for the Synthesis of Maltose-Modified Dense Shell Glycodendrimers. The appropriate dendrimer, D(+)-maltose monohydrate (20 eq. in free amino groups), and borane−pyridine complex (solution 8 M, 40 equiv in free amino groups) were taken up in a sodium borate buffer (30 mL, 0.1 M). The reaction mixture was stirred at 50 °C for 7 d. The crude solution was purified by dialysis toward deionized water for 3 d. Then, the water was removed by freeze-drying to obtain the products as white cottony materials.

mPPI-G4-AdaA. **mPPI-G4-AdaA** was obtained from **PPI-G4-AdaA** (0.18 g, 0.042 mmol) as white fluffy material (0.78 g, yield 85%). ^{1}H



NMR (500 MHz, D_2O): 1.3−2.0 (a, d, g, j, m), 1.68 (z), 1.94 (x), 2.07 (y), 2.3−3.3 (b, c, e, f, h, i, k, l, n, 1′), 3.3−4.4 (2−6, 2′-6′), 4.9−5.3 ppm (1) (Figure SI-1, Supporting Information). ^{13}C NMR (125 MHz, D_2O): 22−26 (d, g, j, m in I), 28.0 (m in II), 32.2 (y), 39.1 (z), 39.6 (n in II), 44.9 (x), 52−57 (b, c, e, f, h, i, k, l, n in I, w), 59−61 (1′), 63.3 (6), 65.2 and 65.6 (6′), 70−71.5 (2′), 72.3 (4), 74−80 (2, 3, 3′, 5, 5′), 83−86 (4′), 103.5 (1), 162.3 ppm (v) (Figure SI-3). IR: 3320 (OH), 2918 (CH, CH_2), 1646 (C═O), 1558 (N−H, NH_2), 1019 cm^{-1} (C−O). LILBID MS: $C_{876}H_{1680}N_{66}O_{544}$ (21830 g/mol^{-1} relating to 54 maltose units and 4 adamantyl urea moieties connected to PPI-G4); m/z = top of the peak of about 21760 (M^-) (Figure SI-7, Supporting Information). The number of maltose units in **mPPI-G4-AdaA** was calculated by LILBID MS and confirmed from the ^{1}H NMR spectrum recorded in D_2O. The intensities of the signals at 1.3−2.1 ppm and at 4.9−5.3 ppm were evaluated taking into account 27.8 terminal NH_2 groups and 4.2 adamantyl groups as determined as number-average for the precursor.

Synthesis of Adamantyl-Modified Maltose Open Shell Glycodendrimer (0.5mPPI-G4-AdaA). **PPI-G4-AdaA** (0.10 g, 0.023 mmol), D-(+)-maltose monohydrate (0.23 g, 0.64 mmol, 1 equiv in free amino groups), and borane−pyridine complex (0.17 mL, solution 8 M) were taken up in a sodium borate buffer (30 mL, 0.1 M). The reaction solution was stirred at 50 °C for 7 d. The crude product was purified by dialysis toward deionized water for 3 d. Then, the water was removed by freeze-drying to obtain the product **0.5mPPI-G4-AdaA** as white fluffy material (0.21 g, yield 82%). The number of maltose units in **0.5mPPI-G4-AdaA** was calculated from the ^{1}H NMR spectrum recorded in D_2O. The intensities of the signal regions at 1.3−2.1 ppm and at 4.9−5.3 ppm were evaluated taking into account 27.8 terminal NH_2 groups and 4.2 adamantyl groups as determined as number-average for the precursor. ^{1}H NMR (500 MHz, D_2O): 1.3−2.0 (a, d, g, j, m), 1.68 (z), 1.94 (x), 2.07 (y), 2.3−3.2 (b, c, e, f, h, i, k, l, n, 1′), 3.3−4.4 (2−6, 2′-6′), 4.9−5.3 ppm (1) (Figure SI-1, Supporting Information). ^{13}C NMR (125 MHz, D_2O): 23−28 (d, g, j, m in I, II and IV), 28.5 (m in II), 32.2 (y), 39.1 (z), 40.3 (n in II), 41.0 (n in IV), 44.9 (x), 48−50 (n in III), 52−59 (b, c, e, f, h, i, k, l, n in I, w, 1′), 63.2 (6), 65−67 (6′), 69−80 (2−5, 2′, 3′, 5′), 83−85.5 (4′), 99.3 and 103.4 (1), 162.3 and 164.0 ppm (v) (Figure SI-4, Supporting

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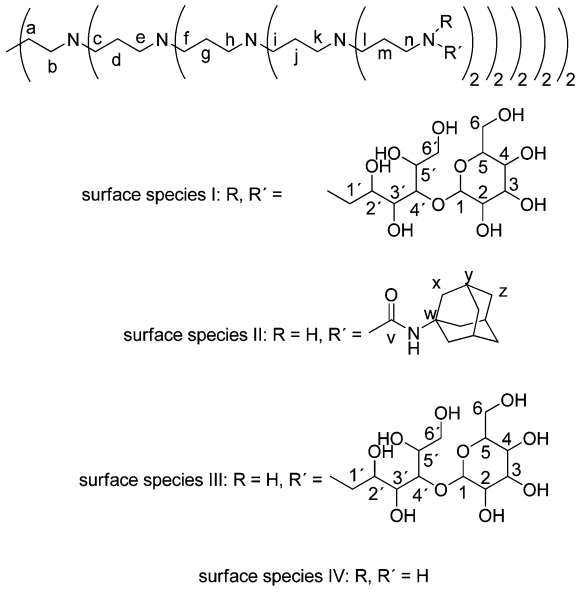
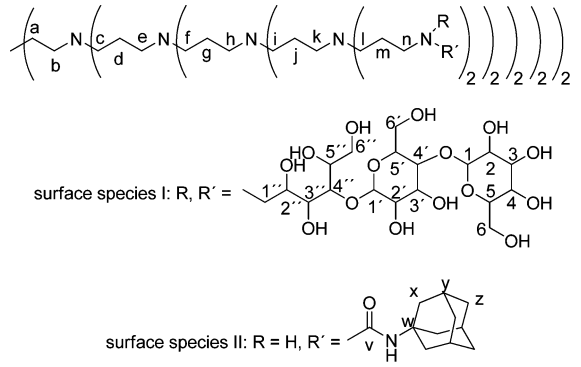


Information). IR: 3345 (OH), 2913, 2849 (CH, CH_2), 1643 (C═O), 1555 (NH, NH_2), 1025 cm^{-1} (C−O).

Synthesis of Adamantyl-Modified Maltotriose Glycodendrimer (m3PPI-G4-AdaA).



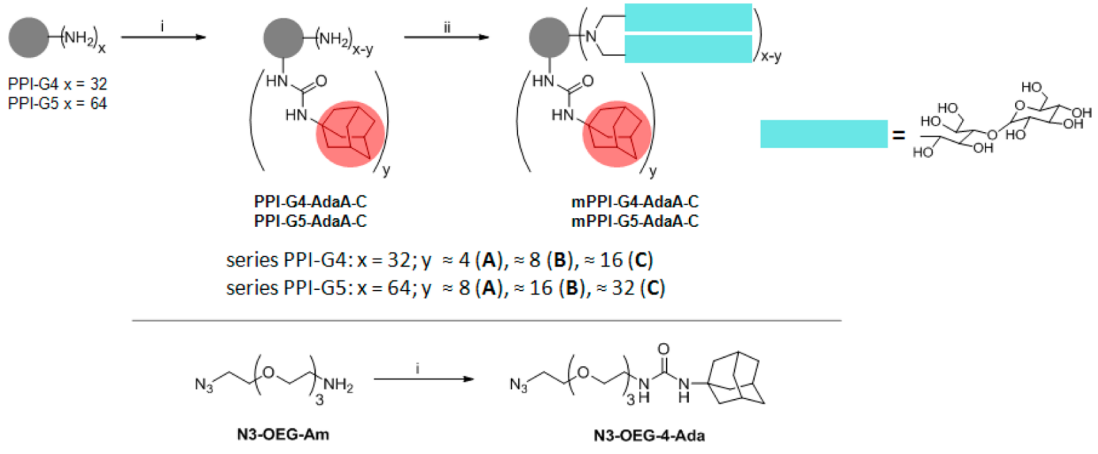
PPI-G4-AdaA (0.10 g, 0.023 mmol), maltotriose hydrate (6.69 g, 13.3 mmol, 20 equiv in free amino groups), and borane−pyridine complex (1.66 mL, solution 8 M) were taken up in a sodium borate buffer (30 mL, 0.1 M). The reaction solution was stirred at 50 °C for 7 d. The crude product was purified by dialysis toward deionized water for 3 d. Then, the water was removed by freeze-drying to obtain the product **m3PPI-G4-AdaA** as white fluffy material (0.56 g, yield 83%). The number of maltotriose units in **m3PPI-G4-AdaA** was calculated from the ^{1}H NMR spectrum recorded in D_2O. The intensities of the signals at 1.3−2.1 ppm and at 4.9−5.5 ppm were evaluated taking into account 27.8 terminal NH_2 groups and 4.2 adamantyl groups as determined as number-average for the precursor. ^{1}H NMR (500 MHz, D_2O): 1.3−2.0 (a, d, g, j, m), 1.68 (z), 1.94 (x), 2.07 (y), 2.3−3.3 (b, c, e, f, h, i, k, l, n, 1″), 3.3−4.4 (2−6, 2′-6′, 2″-6″), 4.9−5.3 (1), 5.3−5.5 ppm (1′) (Figure SI-1, Supporting Information). ^{13}C NMR (125 MHz, D_2O): 23−26 (d, g, j, m in I), 28.1 (m in II), 32.2 (y), 39.1 (z), 40.3 (n in II), 44.9 (x), 52−57 (b, c, e, f, h, i, k, l, n in I, w), 59−61 (1″), 63.2 (6, 6′), 65.1 and 65.6 (6″), 70−81 (2−5, 2′-5′, 2″, 3″, 5″), 82−87 (4″), 102.7 (1′), 103.3 (1), 162.3 ppm (v) (Figure SI-5, Supporting Information). IR: 3267 (OH), 2916 (CH, CH_2), 1647 (C═O), 1559 (N−H, NH_2), 1021 cm^{-1} (C−O).

3. RESULTS AND DISCUSSION

3.1. Synthesis. The introduction of adamantyl groups and sugar molecules on the periphery of the dendrimers was carried out by versatile reactions (Scheme 1).[49]

The first step of synthesis was the introduction of adamantyl groups through an urea bond. Thus, PPI-G4 (32 free amino groups on the periphery) or PPI-G5 (64 free amino groups on the periphery) was reacted with 1-adamantyl isocyanate in different molar ratios (12.5% (**AdaA**), 25% (**AdaB**), and 50% (**AdaC**) in free amino groups) in chloroform to obtain two series of compounds **PPI-G4-AdaA−PPI-G4-AdaC** and **PPI-G5-AdaA−PPI-G5-AdaC**. This resulted in a good decoration of the PPI-G4 and PPI-G5 dendrimer surface with different amounts of adamantyl urea groups. In fact, the content of adamantyl units as determined by ^{1}H NMR analysis is in line with the stoichiometry used (Table 1). Furthermore, the same reaction was used to synthesize the model compound **N3-OEG-4-Ada** that has been used as reference in the NMR studies (Scheme 1).

Scheme 1. Synthesis of Adamantyl-Modified Maltose Dense Shell Glycodendrimers mPPI-Gx-AdaA-C and Model Compound *N*-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-*Ń*-adamantyl Urea (N3-OEG-4-Ada).[a]



[a]Reagents: (i) 1-adamantyl isocyanate, $CHCl_3$; (ii) maltose, BH_3·Pyr, 0.1 M sodium borate solution. An accurate calculation of the derivatization degrees is reported in Table 1.

Table 1. Characteristics of Adamantyl-Modified Dendrimers

entry	number of adamantyl groups (degree of substitution)	number of sugar units: theor[a]	number of sugar units: expt[b]	M_n (g/mol)
PPI-G4-AdaA	4.2 (13%)[c]	–	–	4258[d]
PPI-G4-AdaB	8.3 (26%)[c]	–	–	4985[d]
PPI-G4-AdaC	16.6 (52%)[c]	–	–	6456[d]
PPI-G5-AdaA	8.3 (13%)[c]	–	–	8639[d]
PPI-G5-AdaB	17.9 (28%)[c]	–	–	10341[d]
PPI-G5-AdaC[49]	34.6 (54%)[c]	–	–	13300[d]
mPPI-G4-AdaA	4 (12.5%)[b]	56	54[b]	21760[e]
mPPI-G4-AdaB	8 (25%)[b]	48	44[b]	19188[e]
mPPI-G4-AdaC	16 (50%)[b]	32	29[b]	15865[e]
mPPI-G5-AdaA	8.3 (13%)[f]	112	112[g]	45185[h]
mPPI-G5-AdaB	15 (23%)[b]	98	98[b]	41806[e]
mPPI-G5-AdaC[49]	33 (52%)[b]	62	53[b]	30333[e]
0.5mPPI-G4-AdaA	4.2 (13%)[f]	28	21[g]	11110[h]
m3PPI-G4-AdaA	4.2 (13%)[f]	56	51[g]	29168[h]

[a]Calculated assuming 50% (**0.5m**) and 100% (**m**, **m3**) conversion of remaining NH groups with sugar. [b]Calculated from LILBID mass spectrometry data. [c]Calculated from ^{1}H NMR signal integrals, estimated relative error: ± 5%. [d]Calculated from M_n of PPI-G4 and PPI-G5 and degree of substitution. [e]m/z value of center of the peak obtained by LILBID mass spectroscopy. [f]Value calculated for the precursor by ^{1}H NMR. [g]Calculated by ^{1}H NMR based on the number of adamantyl groups determined for the precursor by ^{1}H NMR, estimated relative error: ± 5%. [h]Calculated from M_n of PPI-G4 and PPI-G5 and the degree of substitutions with adamantyl groups and maltose (or maltotriose) units determined by ^{1}H NMR spectroscopy.

At this point, the adamantyl modified dendrimers were reacted in a 0.1 M aqueous solution of sodium borate with an excess of maltose (Mal) in the presence of borane-pyridine complex as reducing agent to replace the free amino groups of the dendrimer surface (Scheme 2). From this reaction, a series of glycosylated dendrimers with almost complete conversion of $-NH_2$ groups to $-NMal_2$ groups were obtained (**mPPI-**). In addition, two other glycodendrimers, **0.5mPPI-G4-AdaA** and **m3PPI-G4-AdaA**, were synthesized under similar reaction conditions (Scheme 2). The first was obtained by reductive amination of **PPI-G4-AdaA** using maltose in equimolar ratio with the free amino groups. A large excess (20:1) of maltotriose was used for the glycosylation of **PPI-G4-AdaA** in order to obtained highly glycosylated **m3PPI-G4-AdaA.**

0.5mPPI-G4-AdaA is an open shell glycodendrimer in which the primary amino groups are partially replaced by maltosylated units. Thus, its structure is characterized by a predominance of secondary amino groups (−NHMal) as surface groups but also nonreacted primary amino groups and double-reacted tertiary amino groups ($-NMal_2$) were proved by ^{13}C NMR in accordance with a largely random maltosylation reaction. In opposite, the use of maltose or maltotriose in excess results in dense shell glycodendrimers in which almost all terminal amino groups on the periphery are substituted with two sugar molecules. Thus, the dendrimer surface is characterized by the presence of a dense hydrophilic shell bearing hydrophobic imperfections constituted by the lipophilic adamantyl groups (Ada). The structures of these glycodendrimers were characterized by NMR analysis (presence of adamantyl groups and sugar units) and through the evaluation of molecular weights obtained by LILBID-mass spectrometry.

3.2. Interaction of Dendrimers with β-CD. In the first part of this work the host−guest interaction between monomeric β-CD and adamantyl groups directly linked on the glycosylated dendrimers was studied by ^{1}H NMR spectroscopy to prove the possibility of the host−guest interaction on structures highly congested by the presence of different oligosaccharide open and dense shells. As well documented in literature, ^{1}H chemical shift changes of the adamantane protons can be used to evaluate the ability of the β-CD to interact with the adamantyl moiety. Thus, methylene and methine protons of the adamantyl groups shifted toward downfield as a result of the complexation in the hydrophobic cavity of the β-CD.[11,50,51]

For our studies, the glycodendrimers were dissolved in D_2O (1 mM in Ada units) and increasing amounts of 10 mM β-CD solution in D_2O were added. Generally, the increasing β-CD/Ada ratio results in variation of chemical shift and line width for all Ada signals. As reference value for the observed chemical shift changes the nonglycosylated **PPI-G4-AdaA** was also titrated in this way. For this dendrimer with the lowest sterical hindrance around Ada units, a significant excess of β-CD should result in an almost complete complexation of these units (Figure SI-8, Supporting Information). In fact, starting with a 2.5-fold excess of β-CD the chemical shifts of $H_x - H_z$ (numbering according formulas in Experimental Section) remain constant with $\Delta\delta_{max}$ = +0.071 ppm (H_x), +0.147 ppm (H_y), and +0.057 ppm (H_z), respectively. For all titrations of glycosylated dendrimers the maximum observed chemical shift effects were smaller than the values determined for **PPI-G4-AdaA** except for maltosylated PPI-G4 dendrimers in solutions saturated in β-CD. For each titration step that is addition of a certain amount of β-CD, the chemical shift change for the Ada signals compared to the chemical shift without β-CD was determined ($\Delta\delta$). The correlation of the observed $\Delta\delta$ values to the observed $\Delta\delta_{max}$ values (obtained in the titration experiment of **PPI-G4-AdaA**) gives the molar fraction of complexed Ada units, assuming that $\Delta\delta_{max}$ is not affected by a surrounding of terminal oligosaccharide groups. This seems to be reasonable because the interactions are within the β-CD cavity and so screened from the surrounding. However, the degree of complexation itself should be sensitive to the surrounding.

In the complexation studies, only the chemical shift change of the signal attributed to the methine protons (H_y) of Ada unit was evaluated by two reasons. First of all, the largest chemical shift changes are observed for these protons; i.e., they are most sensitive to interactions within the β-CD cavity. Second, the signals of the methylene protons (H_x, H_z) of Ada units significantly overlap with a broad signal of PPI methylene protons resulting in lower accuracy in $\Delta\delta$ determination. For a comparative study the **PPI-G4-AdaA** scaffold with four of the 32 end groups substituted by Ada units was selected. Figure 1 depicts the dependency of the molar fraction of complexed Ada on the ratio β-CD/Ada up to 3.5 for the parent dendrimer **PPI-G4-AdaA** (reference), the open shell glycodendrimer **0.5mPPI-G4-AdaA**, and the dense shell glycodendrimers **mPPI-G4-AdaA**, and **m3PPI-G4-AdaA**.

The comparison of the complexed molar fraction in dependence on the ratio β-CD/Ada (Figure 1) shows that the partial decoration with maltose molecules, as it occurs in the open shell dendrimer **0.5mPPI-G4-AdaA**, produces only small differences in the complexation behavior (compare **0.5mPPI-G4-AdaA** with parent dendrimer **PPI-G4-AdaA**, ^{1}H NMR titrations in Figures SI-9 and SI-10, Supporting

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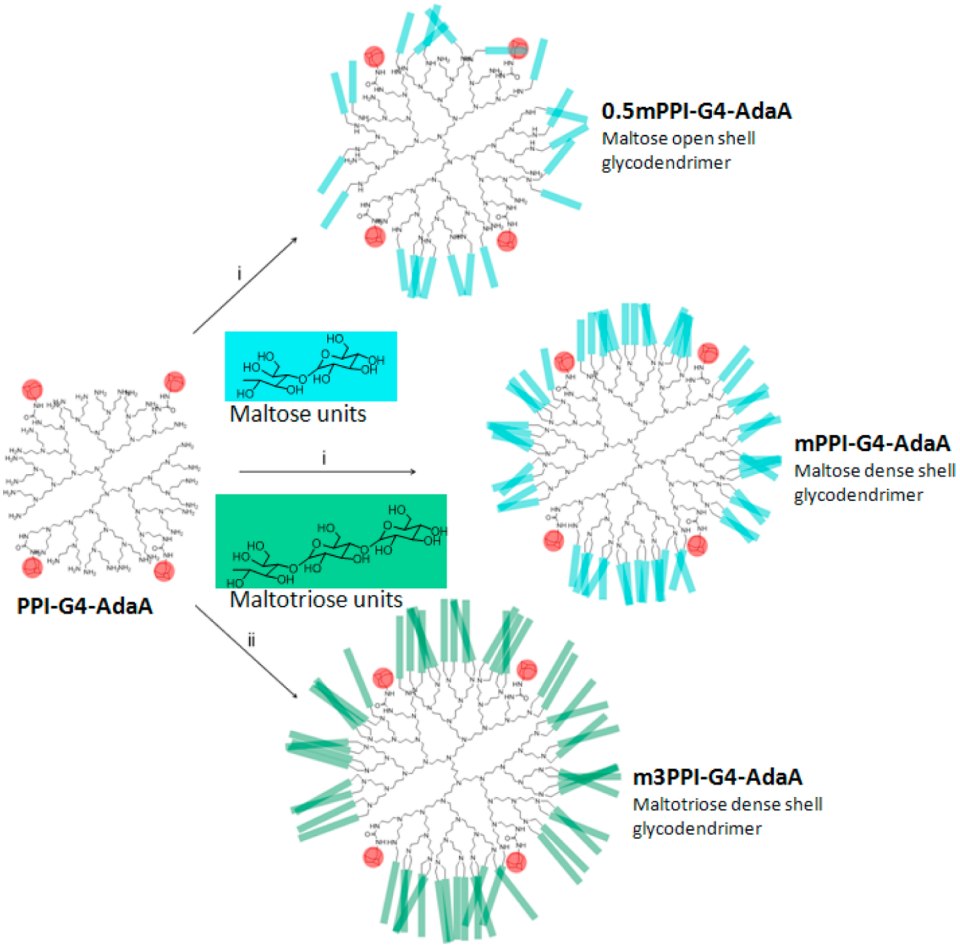
Scheme 2. Synthesis of the Adamantyl-Modified Glycodendrimers 0.5mPPI-G4-AdaA, mPPI-G4-AdaA. and m3PPI-G4-AdaA.[a]



[a]Reagents: (i) maltose (for **0.5mPPI-G4-AdaA**: 1 equiv in free amino groups, for **mPPI-G4-AdaA**: 20 equiv in free amino groups), BH_3·Pyr, 0.1 M sodium borate solution; (ii) maltotriose, BH_3·Pyr, 0.1 M sodium borate solution.

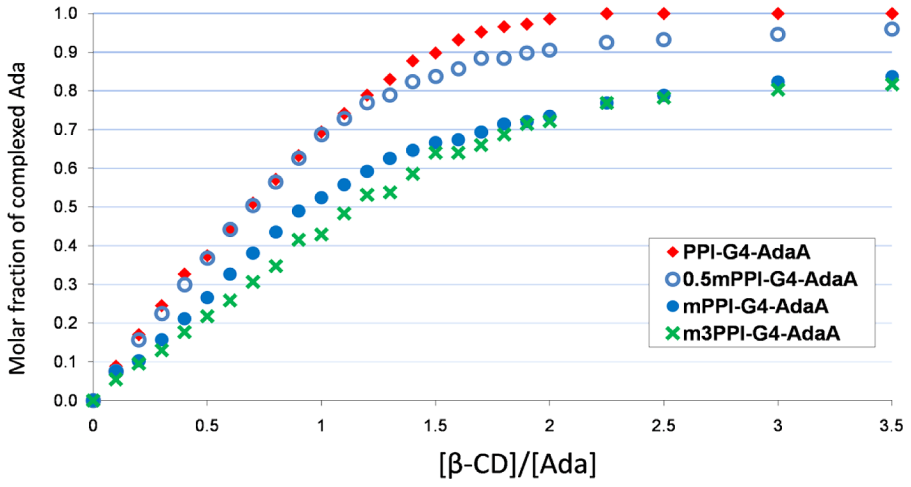


Figure 1. Molar fraction of complexed Ada units of **PPI-G4-AdaA** (◇), **0.5mPPI-G4-AdaA** (○), **mPPI-G4-AdaA** (●) and **m3PPI-G4-AdaA** (×) as a result of increased molar ratio β-CD/Ada.

Information). This similarity is particularly stringent up to β-CD/Ada =1.3 corresponding to about 80% of complexed adamantanes. A slightly divergent behavior is observed at higher β-CD/Ada values with **0.5mPPI-G4-AdaA** curve showing a lower complexation at the same degree of titration that is compatible with the additional steric hindrance. The inclusion of the adamantane in the dense maltose shell structure of **mPPI-G4-AdaA** decreases the ability of β-CD to bind the adamantane units in the glyco-surface (^{1}H NMR titration in Figure SI-10, Supporting Information). The further increase of steric hindrance on the surface due to the use of maltotriose in **m3PPI-G4-AdaA** causes only a small difference

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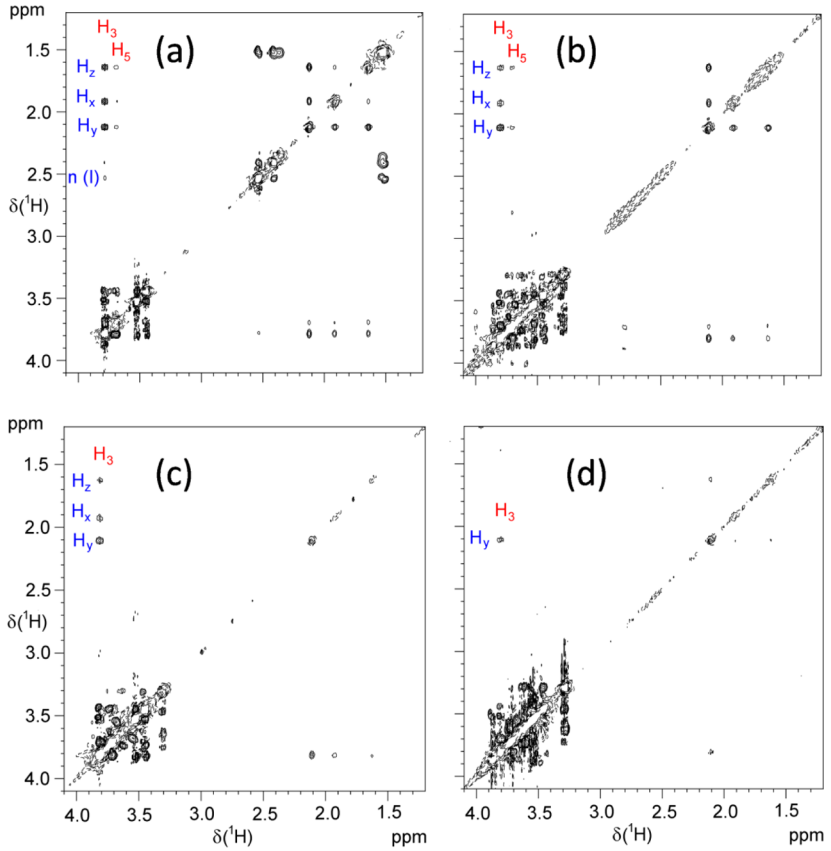

Figure 2. $^1H-^1H$ ROESY spectra: (a) **PPI-G4-AdaA**, (b) **0.5mPPI-G4-AdaA**, (c) **mPPI-G4-AdaA**, and (d) **m3PPI-G4-AdaA**. All spectra were recorded in D_2O with a ratio β-CD/Ada = 2.

in accessibility to adamantane in comparison with **mPPI-G4-AdaA** (^1H NMR titration in Figure SI-11, Supporting Information). It is obvious that steric hindrance within the dense shell of the glycosylated PPI-G4 dendrimers **mPPI-G4-AdaA** and **m3PPI-G4-AdaA** hampers access to a certain degree but more than 80% of the Ada units are still accessible for complexation at β-CD/Ada =3 and almost complete Ada complexation is observed in saturated β-CD solution.

In order to understand the observed Ada-β-CD interaction from a qualitative point of view, $^1H-^1H$ rotating frame Overhauser effect spectroscopy (ROESY) measurements were performed for each dendrimer of the **PPI-G4-AdaA**-based series (4 mM in adamantyl unit) in the presence of β-CD at ratio β-CD/Ada =2 (Figure 2). Cross-peaks between the adamantyl protons $H_x - H_z$ and the β-CD protons H_3 and H_5 located in the more hydrophobic cavity are a prove of Ada/β-CD complexation. In fact, **PPI-G4-AdaA** shows a clear interaction of the adamantyl protons with protons H_3 and H_5 of β-CD and a weak interaction of propylamine terminal groups with H_3 proton of β-CD, too (Figure 2a). The later interaction was confirmed by a ROESY experiment on a PPI-G4/β-CD mixture. This competitive interaction may explain the necessity of a ~2.25-fold excess of β-CD to obtain full complexation of adamantane in the structure without maltose. A pitfall which can result in misinterpretation of ROESY cross-peaks is the signal overlap of maltose and β-CD signals. However, a ROESY spectrum of **mPPI-G4-AdaA** without β-CD does not show any cross-peak between maltose and adamantyl signals.

Thus, the intense cross-peaks between the adamantyl protons and protons H_3 and, with lower intensity, H_5 of β-CD in the ROESY spectrum of **0.5mPPI-G4-AdaA** confirm the formation of the inclusion complex (Figure 2b). A similar effect was observed for **mPPI-G4-AdaA** and **m3PPI-G4-AdaA** proving formation of Ada/β-CD complex. The higher decrease in intensity for the H_5 compared to the H_3 cross-peaks suggests that the adamantyl moiety penetrates from the wider side of the β-CD ring and the depth of penetration of the adamantyl moiety decreases with increasing steric hindrance due to higher density of the sugar shell. However, several effects can cause the observed loss in intensity of cross-peaks (i.e., decreasing signal/noise ratio and increasing rotating frame spin–lattice relaxation rate) and, thus, a more quantitative interpretation is not intended. In any case, it seems important to emphasize that the presence of attached molecules of maltose or maltotriose on **PPI-G4-AdaA** not completely prevents the ability of β-CD to bind to the dendrimer surface.

Figure 3 depicts a comparison of the ^1H NMR spectra of the dense shell glycodendrimers **mPPI-G4-AdaA-C** and **mPPI-G5-AdaA-C** dendrimers with different amount of adamantyl units on the surface (Scheme 1) after complexation with β-CD (1:2 molar ratio Ada/β-CD). Within the **mPPI-G4-AdaA-C** series the fraction of complexed Ada, as determined from $\Delta\delta$ of H_y and $\Delta\delta_{max}$ = +0.147 ppm, decreases from 75% (**A**) to ~65% (**C**). This slight decrease indicates comparable accessibility of Ada units up to this loading. Nevertheless, an increased "dynamic" steric hindrance due to complexed adamantanes is obvious. Saturating the solution with β-CD results in almost the same chemical shifts for H_x-H_z, proving full complexation also for **mPPI-G4-AdaC** bearing at this stage 17 β-CD-complexed Ada units and 15 double-maltosylated amino groups on the

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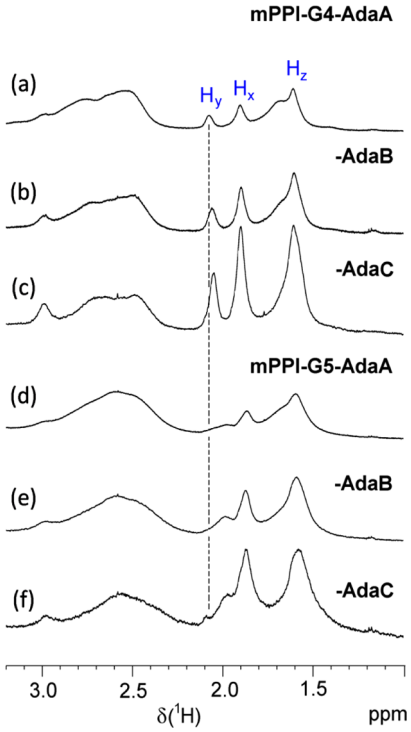

Figure 3. ^{1}H NMR spectra of dense shell glycodendrimers of the series **mPPI-G4-AdaA-C** (a–c) and **mPPI-G5-AdaA-C** (d–f) with β-CD at a β-CD/Ada ratio of about 2.

surface. Remarkably, the Ada signals become narrower for all compounds with complexation which is most obvious for **mPPI-G4-AdaC**. Here, Ada units with restricted mobility probably in hydrophobic clusters were mobilized by inclusion in the β-CD cavity. For the higher generation **mPPI-G5-AdaA-C** series, β-CD addition results in a broad signal hampering the determination of accurate $\Delta\delta$ values but the β-CD loading is obviously lower than for the **mPPI-G4-AdaA-C** series. As with increasing dendrimer generation the shell density increases,[52] Ada complexation is more demanding for G5 generation, and to obtain the same loading a higher excess of β-CD is required. However, also in a saturated β-CD solution the final $\Delta\delta$ values are smaller than for the corresponding **mPPI-G4** samples indicating incomplete Ada complexation. This is in accordance with results of Reinhoudt et al. showing that in contrast to adamantyl terminated PPI-G4, in adamantyl terminated PPI-G5 not all 64 Ada groups at the surface can be complexed by β-CD for steric reasons.[30,31]

The NMR studies on Ada complexation by β-CD conclusively reveal the graded effect of steric hindrance on the surface modulated by the number and type of saccharide molecules and the number of Ada units. Thus, the accessibility of Ada decreases at the same Ada/β-CD ratio with increasing degree of maltosylation, size of the saccharide molecule and Ada substitution. The retarding effect on complexation is more pronounced for fifth generation PPI.

3.3. Interaction of Dendrimers with *poly*-β-CD. β-Cyclodextrin is reported in many studies as a component of polymeric materials. Within the polymeric structures, the β-CD units can be a pendant group or part of the polymeric skeleton. In both cases a large number of β-CD units is located in one macromolecule making it an interesting candidate for potential applications such as controlled release of drugs, inclusion of lipophilic guests, chromatographic technique, and more.[18,19]

Furthermore, the β-CD in polymeric form can increase the stability constant of the inclusion process of lipophilic guests.[53,54] On the basis of the ability to attach the β-CD on the dendrimeric surface by host–guest interaction with the adamantyl moiety (as described before), we decided to investigate the interaction of the dendrimers **PPI-G4-AdaA**, **0.5mPPI-G4-AdaA**, **mPPI-G4-AdaA**, and **m3PPI-G4-AdaA** with a model polymer containing β-CD units. A commercially available water-soluble polymer (*poly*-β-CD) based on the reaction of β-CD with epichlorohydrin (EP) was chosen for this study.[47] The polymer having a EP/β-CD ratio of 6.8 (determined as described in Experimental Section) is characterized by β-CD units randomly trapped between oligomeric 2-hydroxypropyl ether chains (Figure 4). The

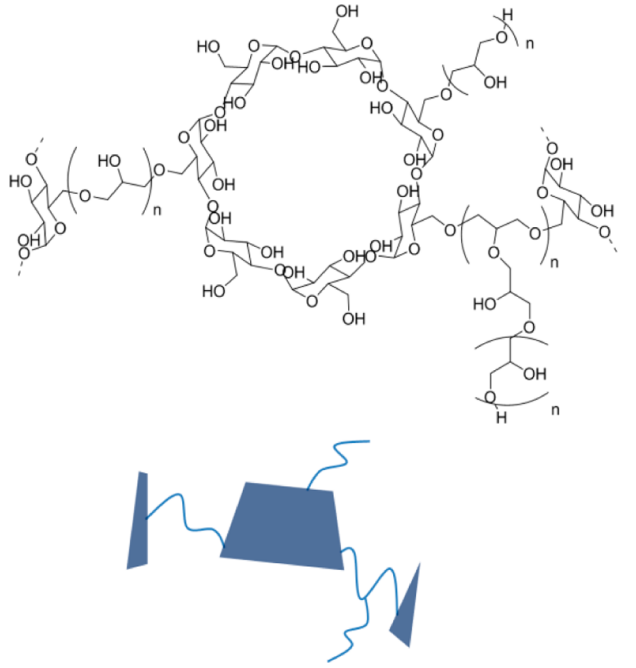

Figure 4. Structure of the random polymer *poly*-β-CD and a cartoon representation of it.

random reaction results in a broad molecular weight distribution (2.000–15.000 g/mol) and a low average molecular weight of 3.500 g/mol that ensure a good solubility in water (>0.25 g/mL). The β-CD content was found to be 63.5% in weight by ^{1}H NMR analysis (see Experimental Section) and this value was used for the calculation of the concentration of β-CD units in *poly*-β-CD solutions used in the NMR and DLS experiments.

The ^{1}H NMR complexation studies on **PPI-G4-AdaA**, **0.5mPPI-G4-AdaA**, **mPPI-G4-AdaA**, and **m3PPI-G4-AdaA** with *poly*-β-CD adopt the method described for the host–guest titration with β-CD (stock solution 1 mM in adamantyl units; gradual addition of a *poly*-β-CD solution 10 mM in β-CD units; solvent D_2O; ^{1}H NMR titration in Figure SI-12–15, Supporting Information). The addition of *poly*-β-CD to the dendrimer solutions results in a particular effect in the ^{1}H NMR spectra (Figure 5a,b). Namely, the signals attributed to noncomplexed adamantanes ($H_{(x,y,z)}$) does not undergo a progressive shift to downfield, as it occurred with the addition of monomeric β-CD. In the case of the *poly*-β-CD addition, these adamantyl signals decrease in intensity and simultaneously three other signals appear downfield in the ^{1}H NMR

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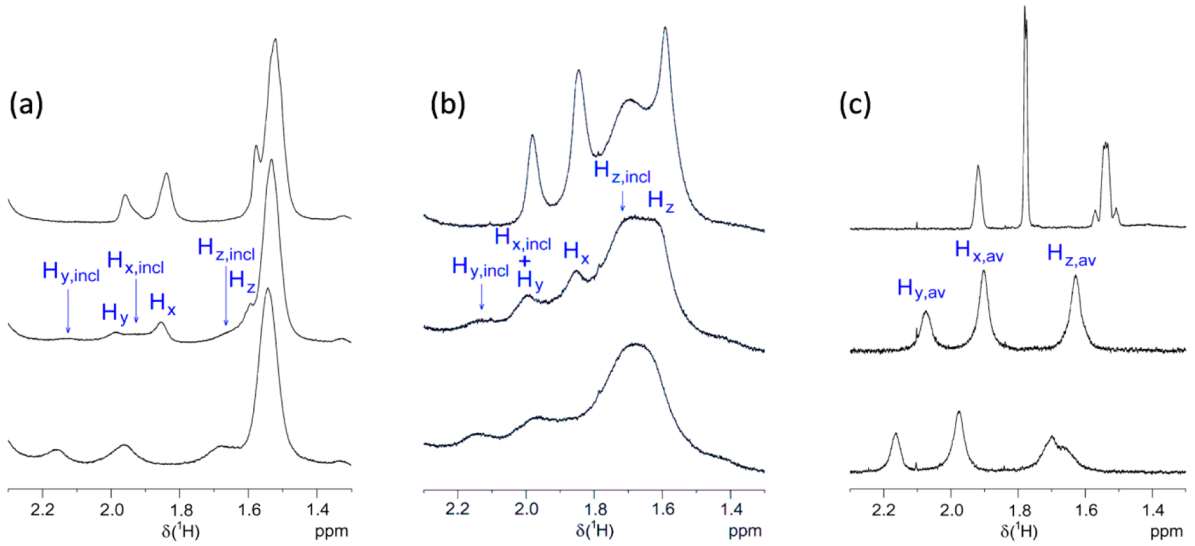

Figure 5. Selected ^{1}H NMR spectra of titrations of **PPI-G4-AdaA** (a), **mPPI-G4-AdaA** (b) and **N3-OEG-4-Ada** (c) with *poly*-*β*-CD. Top spectra were obtained from D_2O solutions 1 mM in adamantyl units. The middle traces give spectra obtained at *poly*-*β*-CD/Ada rations of 0.7 (a), 1.5 (b), and 0.5 (c). The bottom spectra were obtained at *poly*-*β*-CD/Ada rations of 2 (a, c) and 4 (b).

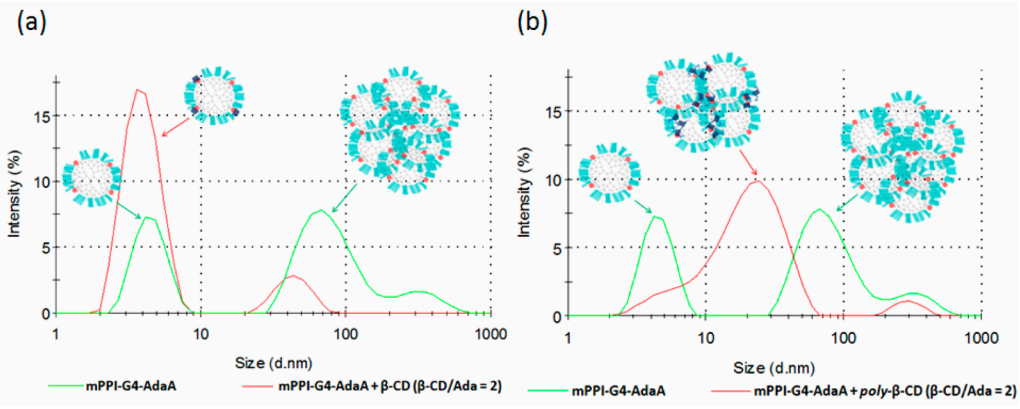

Figure 6. DLS analysis of the dendrimers **mPPI-G4-AdaA** with (a) *β*-CD and (b) *poly*-*β*-CD All spectra were recorded at concentration of 1 mM in adamantyl unit and *β*-CD or *poly*-*β*-CD was added at ratios *β*-CD/Ada =2. The volume plots of this DLS study are presented in the Supporting Information (Figure SI-17).

spectra (middle traces in Figure 5a,b) at signal positions corresponding to Ada units included in the *β*-CD cavity ($H_{(x,y,z),incl}$). These signals increase in intensity with increasing amount of *poly*-*β*-CD proving the supramolecular interactions between the Ada units on the PPI surface and the *β*-CD units present in *poly*-*β*-CD (bottom traces in Figures 5a,b). Generally, there is a dynamic equilibrium between non-complexed and complexed Ada units due to the fact that the noncomplexed Ada units can be included in a host−guest complex but can also be released. In both states the Ada protons have characteristic chemical shifts. The Ada complexes with monomeric and polymeric *β*-CD differ in the exchange rate between both states. Whereas the process is fast on the NMR time scale for all complexes with monomeric *β*-CD resulting in an averaged signal for free and complexed Ada units, the process is slow for interactions with *β*-CD units in the *poly*-*β*-CD backbone. The reduced exchange rate can be rationalized in terms of the lower diffusion rate of *poly*-*β*-CD compared with monomeric *β*-CD, the restricted mobility of *β*-CD units in *poly*-*β*-CD, and the multivalent character of the *poly*-*β*-CD (the interaction of the first *β*-CD unit with the available adamantyl moiety increases the local concentration of *β*-CD units). This is not contrary to the averaged signals observed for the complexation of a low-molecular-weight model compound **N3-OEG-4-Ada** with terminal adamantyl unit by *poly*-*β*-CD (Figure 5c, ^{1}H NMR titration in Figure SI-16, Supporting Information). Similar to monomeric *β*-CD, the small model compound can form and leave the complex very fast. It should be noted that almost complete Ada complexation is reached already at 1:1 *β*-CD/Ada ratio because of absent steric hindrance, which in contrast hampers the complexation of Ada units bonded on the PPI-surface. Obviously, the interactions between two large molecules with lowered diffusion rate and the statistical effect cause increased lifetime of the complex. Thus, both states of Ada units can be observed by their characteristic ^{1}H signals and their ratio could give the degree of complexation. In practice, this is hampered by broad and overlapping signals (Figure 5). Contrary to Ada complexation with monomeric *β*-CD, the line width of Ada signals increases by complexation with *poly*-*β*-CD most probably by restricted mobility in the dendrimer/*poly*-*β*-CD aggregate. Nevertheless, at sufficient high excess of *poly*-*β*-CD a complete

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or at least high degree of Ada complexation can be concluded (bottom traces in Figure 5a,b).

The complete complexation of adamantane is reached at different concentrations of β-CD unit in relation to the steric hindrance present on the dendrimer surfaces. In fact, parent dendrimer **PPI-G4-AdaA** seems to reach an apparently total complexation at a β-CD/Ada ratio of ca. 1.75. β-CD/Ada ratios of 2 and 3.5 are necessary to obtain a complete apparent complexation of the maltose open shell glycodendrimer **0.5mPPI-G4-AdaA** and the maltose dense shell glycodendrimer **mPPI-G4-AdaA** respectively, while in the maltotriose dense shell glycodendrimer **m3PPI-G4-AdaA** a large excess of β-CD unit (β-CD/Ada =5) is not sufficient to complete the complexation.

Thus, the entrapment of β-CD in a polymeric structure does not prevent the interaction with the adamantanes on the dendrimeric surface, but (as it happened with the monomeric β-CD) a modulation of the complexation ability is present that decreases as a result of the steric hindrance caused by the sugar shell. On the other hand, the use of *poly*-β-CD seems to provide greater complexation stability and can be used to induce controlled aggregation between various macromolecules in a heterogeneous system consisting of ordered polymers (dendrimers), and a random polymer (*poly*-β-CD).

3.4. Host–Guest Interaction Behavior of mPPI-G4-AdaA Investigated by DLS. In order to obtain information about the influence of host–guest interactions on the supramolecular behavior (i.e., aggregation) of these adamantyl-functionalized glycodendrimers, **mPPI-G4-AdaA** was evaluated in a preliminary DLS study in the presence of β-CD and *poly*-β-CD. The aim was to clarify whether **mPPI-G4-AdaA** and its host–guest inclusion complexes can form defined supramolecular aggregates as known from other dendritic supramolecular host–guest complexes.[55−57] Therefore, intensity plots from the different **mPPI-G4-AdaA** solutions were analyzed (Figure 6), which should highlight aggregation phenomena of **mPPI-G4-AdaA** in the presence of β-CD or *poly*-β-CD, respectively.

The DLS spectrum of the dense maltose shell dendrimer **mPPI-G4-AdaA** shows two peaks: the first at ca. 68 nm is attributed to a low degree of aggregation, and the second at 4.5 nm is attributed to the isolated macromolecules (PDI = 0.487). The addition of β-CD to the solution of **mPPI-G4-AdaA** (up to ratio β-CD/Ada = 2) results in a breakdown of the aggregates as can be concluded from the almost complete disappearance of the component at 68 nm accompanied by the increase of the nonaggregated component at 4.5 nm (Figure 6a) and the decrease of PDI value to 0.241.

In maltose dense shell glycodendrimers, the substitution of the terminal amino groups of the dendrimeric surface by maltose molecules was assumed to prevent significantly the backfolding of the dendrimeric arms by creating a hydrophilic external shell capable of shielding the hydrophobic core.[58] Since the backfolding effect facilitates the intermolecular packing between dendrimers, it is considered an important factor for the aggregation of dendrimers.[59,60] The substitution with maltose molecules prevents the backfolding and significantly decreases the intermolecular packing between the glycodendrimers and consequently reduces the aggregation phenomenon. In the present adamantyl-modified dendrimers, the weak aggregation of the maltose dense shell glycodendrimer (**mPPI-G4-AdaA**) could be mainly due to weak hydrophobic interactions that occur (at the concentration used in these experiments) as a result of imperfections generated by the presence of the adamantyl moieties in the external maltose shell. These assumptions appear to be supported by the fact that aggregation is significantly depressed as a result of the complexation of the adamantyl groups with β-CD that dynamically reconstructs the external sugar dense shell. In this way, the supramolecular system formed by the dendrimer plus β-CD (Figure 7) shows a very dense saccharide shell comparable to the perfect maltose dense shell of the previously published dendrimers that do not show any aggregation in water.[40]

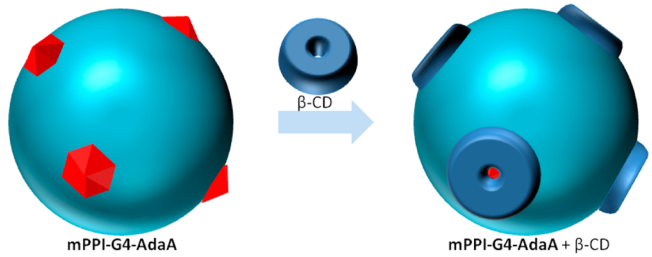

Figure 7. Illustration of the reconstruction of the external sugar dense shell of **mPPI-G4-AdaA** disturbed by few hydrophobic Ada units by adding of monomeric β-CD which covers these defects.

Interestingly, the interaction of *poly*-β-CD (i.e., the addition of *poly*-β-CD to the dendrimer solution up to ratio β-CD/Ada =2) with the maltose dense shell glycodendrimer **mPPI-G4-AdaA** is able to convert both signals of the DLS spectrum of **mPPI-G4-AdaA** (isolated macromolecules at 4.5 nm and weak native aggregates at 68 nm) in a main signal (PDI value =0.159) with an average size dimension of about 25 nm, which we attribute to small supramolecular structures formed as a result of the interaction between *poly*-β-CD and Ada moieties (Figure 6b). This behavior is in line with that observed using the monomeric β-CD. In fact, the dense shell of maltose molecules significantly reduces the intermolecular packing between dendrimers and, as it occurs with the monomeric β-CD, the portion of weak native aggregates present in solution is broken as a result of complexation of adamantyl groups by the β-CD moiety contained in the *poly*-β-CD. The formation of small aggregates combining **mPPI-G4-AdaA** and *poly*-β-CD is probably due to the low molecular weight of the random polymer (that ensures ca. 2−3 β-CD units in each macromolecule of *poly*-β-CD) and the low number of adamantyl units on the dendrimeric surface. These two factors may result in a low cross-linking effect between the macromolecules generating controlled sizes of the supramolecular aggregates in the intensity plot. Moreover, the evaluation of the volume plots (Figure SI-17, Supporting Information) was performed to obtain insight on the frequency of occurrence of supramolecular aggregates after the addition *poly*-β-CD. The analysis showed the preferential organization in isolated supramolecular host–guest inclusion particles. This surprising and unexpected result may give us the chance to fabricate defined supramolecular host–guest inclusion complexes with higher hierarchical supramolecular organization in the future.

3.5. Cell Toxicity Studies. In view of their use in the development of supramolecular systems for healthcare applications as nanoparticles (or nanogels), cellular toxicity studies have been conducted on the isolated components (dendrimers and *poly*-β-CD). In fact, no or acceptable low

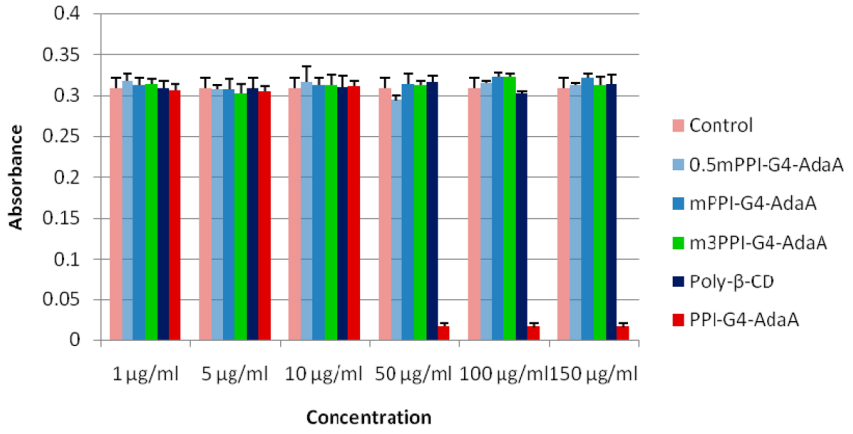

Figure 8. NIH3T3 viability after 24 h of contact with different concentration of **PPI-G4-AdaA**, **0.5mPPI-G4-AdaA**, **mPPI-G4-AdaA**, **m3PPI-G4-AdaA**, and *poly-β*-CD. The results are reported as mean ± SD for each concentration tested in triplicate. No value except those of **PPI-G4-AdaA** at ≥50 μg/mL was statistically different from the control ($p < 0.05$).

levels of intrinsic cytotoxicity is one of the most important requirements for application as a carrier for drugs, genes or biological probes.

Amino-terminated dendrimers (PPI and PAMAM) are reported to have limited potential applications in therapy due to their generation and dose-dependent toxicity profile.[61−63] The functionalization of their peripheral amino groups has been reported to reduce their toxic properties.[43−45,64,65] Therefore, it was important to establish the toxicity profiles of glycodendrimers **0.5mPPI-G4-AdaA**, **mPPI-G4-AdaA**, and **m3PPI-G4-AdaA** in comparison with their precursor **PPI-G4-AdaA**. The toxicity profiles are reported in Figure 8. Treatment with **PPI-G4-AdaA** resulted in a dose-dependent toxic effect on NIH3T3 cells (Figure 8, red bar). In particular, a concentration of 50 μg/mL **PPI-G4-AdaA** drastically reduced the viability of cells, whereas about 100% of cells remained viable following exposure to **PPI-G4-AdaA** at 1, 5, and 10 μg/mL.

The introduction of maltose or maltotriose molecules on the periphery of the **PPI-G4-AdaA** to obtain **0.5mPPI-G4-AdaA** (maltose open shell), **mPPI-G4-AdaA** (maltose dense shell) and **m3PPI-G4-AdaA** (maltotriose dense shell) significantly reduced the toxicity of these compounds (Figure 8), and no decrease in cellular viability was observed for concentrations up to 150 μg/mL.

It was concluded that substitution of amino surface groups of **PPI-G4-AdaA** by maltose or maltotriose molecules, as it occurs in PPI dendrimers without adamantyl moieties,[43−45,65] screens out the (acute) toxicity of these compounds. Furthermore, the adamantyl groups at ca. 13% included in a maltose (or maltotriose) shell on the dendrimers surface are well tolerated by living cells.

The same procedure was used to evaluate the cellular toxicity of *poly-β*-CD. Also for this polymer, at concentrations up to 150 μg/mL, no decrease in cellular viability was observed. In conclusion, from the preliminary studies of cellular toxicity, the biocompatibility of the macromolecules used in the interaction studies with *poly-β*-CD became evident.

4. CONCLUSIONS

The host−guest *β*-CD-Ada interaction is commonly accepted as a powerful tool to build polymeric supramolecular systems. This study shows the application of this particular molecular recognition to dendrimeric systems that expose on the surface adamantyl groups embedded in oligosaccharide shells of variable density. The glycodendrimers were synthesized by two efficient reactions which linked the NH_2 end groups of poly(propyleneimine) dendrimers (fourth and fifth generation) to a variable number of adamantyl moieties by urea bond formation (**PPI-Gx-AdaA**, **-AdaB**, and **-AdaC**; x = 4 or 5) as well as to maltose molecules (or maltotriose in **m3PPI-G4-AdaA**) by reductive amination in the series **mPPI-Gx-AdaA**, **-AdaB**, and **-AdaC** with x = 4 or 5 and **0.5mPPI-G4-AdaA**. The inclusion of the dendrimer-bound adamantyl groups in the hydrophobic cavity of monomeric *β*-CD was evaluated by ^{1}H NMR spectroscopy (host−guest titration and ROESY experiments) to provide evidence of the host−guest interaction on the highly congested structures. The NMR experiments on Ada complexation by monomeric *β*-CD revealed that the accessibility of Ada moieties decreases at the same Ada/*β*-CD ratio with increasing (1) the dendrimer generation, (2) the number and type of oligosaccharide molecules, and (3) the number of Ada units on the surface. Even a dense shell of attached maltotriose molecules hampers but does not prevent the complexation of Ada units by *β*-CD. This is of particular importance in the evaluation of accessibility of surface-bonded molecules in oligosaccharide-modified PPIs. On the basis of the NMR results, fourth generation PPI with 13% substitution of amino groups by adamantyl groups was proved as the most efficient scaffold for further studies. Thus, the four dendrimers **PPI-G4-AdaA** (parent dendrimers), **0.5mPPI-G4-AdaA** (maltose open shell glycodendrimer), **mPPI-G4-AdaA** (maltose dense shell glycodendrimer), and **m3PPI-G4-AdaA** (maltotriose dense shell glycodendrimer), having different number and size of oligosaccharide molecules in the outer shell, were used to investigate the host−guest interaction with a water-soluble *β*-CD based polymer (*poly-β*-CD). Again, the *β*-CD-Ada interaction using *poly-β*-CD was proved by ^{1}H NMR analysis. In contrast to the interaction with monomeric *β*-CD where averaged signals indicate fast exchange between complexed and noncomplexed Ada units on the NMR time scale, individual signals both for complexed and noncomplexed Ada units were observed for the interaction with *β*-CD units in the polymeric scaffold of *poly-β*-CD proving a lower exchange rate. This result was rationalized by the lower diffusion rate of *poly-β*-CD compared with monomeric *β*-CD and a restricted mobility of *β*-CD units in *poly-β*-CD.

In order to understand the effects of *β*-CD-Ada complexation at the macromolecular level, preliminary DLS experiments were

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performed on the selected maltose dense shell dendrimer **mPPI-G4-AdaA** in the presence of an excess amount of β-CD in monomeric and polymeric forms.

The DLS results from intensity plots revealed that isolated **mPPI-G4-AdaA** macromolecules coexist with aggregated species. Interestingly, the addition of monomeric β-CD is capable of suppressing the weak intermolecular connections in **mPPI-G4-AdaA.** On the contrary, the addition of *poly*-β-CD to the solutions of **mPPI-G4-AdaA** leads to the appearance of a new signal (at ca. 25 nm), which was attributed to the formation of aggregate structures consisting of dendrimeric macromolecules bound together by links of *poly*-β-CD through a strong and stable β-CD-Ada interaction. However, these aggregation phenomena were not evident in the volumetric plots of DLS spectra. This suggests that most of the interactions between *poly*-β-CD and the glycodendrimer leads to the formation of isolated supramolecular complexes, consisting in a dendrimeric core branched with macromolecules of *poly*-β-CD.

Therefore, the host−guest interaction-dependent supramolecular behavior of the maltosylated dense shell glycodendrimer bearing a moderate number of adamantyl groups, taken together with the apparent biocompatibility, provides the rational basis for the use of these adamantyl-functionalized glycodendrimers in the rational design of supramolecular systems potentially useful as healthcare materials.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, mass spectroscopy results (LILBID-MS), additional NMR spectra, and additional DLS spectra. This material is available free of charge via the Internet at http://pubs.acs.org/.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: paomar@oneonline.it (M.P.); komber@ipfdd.de (H.K.).

Notes

The authors declare no competing financial interest.

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