

## Supporting information

# Design, synthesis and characterization of fully zwitterionic, functionalized dendrimers

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## Materials & methods

### Materials

Milli-Q water was purified by a Barnsted water purification system, with a resistivity of  $<18.3 \text{ M}\Omega \cdot \text{cm}$ . The reported plasma cleaner was a Diener Femto plasma system. Molecular sieves ( $10 \text{ \AA}$ ) were oven-dried ( $120 \text{ }^\circ\text{C}$ , overnight) prior to use. Sonication steps were performed in an Elmasonic P 30 H ultrasonic unit at 80 kHz. Float-a-lyzer G2 dialysis membranes (VWR) with a 500-1000D DE (default) or 1000-5000D CE (when specifically mentioned) were used for the final purification step. Commercially available reagents were used without purification, unless mentioned otherwise: Poly(propylene imine) dendrimers G2, G3 and G4 (PPI, SyMOChem); ethanol (EtOH, absolute, dried over molecular sieves, Merck); hydrochloric acid (HCl, 37% in water, Acros Organics); dichloromethane (DCM, GPR Rectapur, Fisher Scientific); *n*-hexane ( $\geq 99\%$ , Sigma Aldrich); acetone (Semiconductor grade, Sigma Aldrich); deuterium oxide ( $\text{D}_2\text{O}$ , 99.9 atom% D, Sigma Aldrich); 3-(trimethylsilyl)-1-propanesulfonic acid- $d_6$  sodium salt (TMS salt, 98 atom% D, Sigma Aldrich); formaldehyde (37 wt% in water, 10-15% methanol, Fisher Scientific); formic acid (99%, VWR); *tert*-Butyl bromoacetate (98%, Sigma Aldrich); *tert*-Butyl 2-iodoacetate (Sigma Aldrich); trifluoroacetic acid (Biosolve B.V.); methyl bromoacetate (96%, Sigma Aldrich); methyl 2-iodoacetate (95%, Sigma Aldrich); methyl iodide (99%, stabilized, Fisher Scientific); sodium bromoacetate (98%, Sigma Aldrich); sodium iodoacetate ( $\geq 98\%$ , Sigma Aldrich); sodium 2-bromoethylsulfonate (98%, Sigma Aldrich); sodium 3-bromopropanesulfonate ( $\geq 97\%$ , Sigma Aldrich); propargyl-*N*-hydroxysuccinimidyl ester (NHS-alkyne, Sigma Aldrich); azido-PEG@8-NHS ester (NHS-PEG-azide, Sigma Aldrich); sodium hydroxide (NaOH, 98.5% pellets, Fisher Scientific); sodium sulfate ( $\text{Na}_2\text{SO}_4$ , anhydrous, Fisher Scientific); triethylamine (99%, distilled, on KOH, Fisher Scientific); azide-PEG3-biotin (Sigma Aldrich); sodium L-ascorbate (sodium ascorbate,  $\geq 98\%$ , Sigma Aldrich); copper sulfate pentahydrate ( $\geq 98\%$ , Sigma Aldrich)

## Methods

### *Nuclear magnetic resonance (NMR)*

$^1\text{H}$  NMR measurements were recorded on a Bruker Avance III NMR at 400 MHz,  $^{13}\text{C}$  NMR spectra were recorded at 100 MHz. For the  $^1\text{H}$ - $^{15}\text{N}$  HMBC measurements settings of 600 and 60 MHz were used, respectively, on a 600 MHz Bruker Avance III Ultrashield Plus equipped with a cryoprobe. Chemical shifts are reported in parts per million (ppm), and are referred to the methyl signal of the sodium salt of 3-(trimethylsilyl)-1-propanesulfonic acid- $d_6$  ( $\delta = 0$ ).

### *Infrared (IR)*

IR analyses were performed on a Bruker Tensor 27 spectrometer with platinum ATR accessory.

### *X-ray Photoelectron Spectroscopy (XPS)*

Samples for dendrimer analysis were prepared by concentrating the dendrimers (in milli-Q water) and drop-casting 3  $\mu\text{L}$  of this suspension onto a piece of Si(111) (Siltronix, N-type, phosphorus doped), which was cleaned by rinsing and sonicating for 5 min in semiconductor grade acetone followed by oxygen plasma treatment (Diener electronic, Femto A) for 1 min at 100% power. The dropcast samples were subsequently dried in vacuum overnight before XPS measurements were started. XPS spectra were obtained using a JPS-9200 photoelectron spectrometer (JEOL, Japan) with monochromatic Al-K $\alpha$  X-Ray radiation at 12 kV and 20 mA. The obtained spectra were analyzed using CASA XPS software (version 2.3.16 PR 1.6). In C1s and N1s narrow-range spectra, the positions are set to 285 eV and 400 eV for the C-C and N-C signals, respectively.

### *Gel Permeation Chromatography (GPC)*

The polymer molecular weight and polydispersity index (PDI) were determined using gel permeation chromatography (Agilent G5654A quaternary pump, G7162A refractive index detector), where a PSS SUPREMA Combination medium (P/N 206-0002) 1000 Å single porosity column was employed (0.05%  $\text{NaN}_3$  in milli-Q water as eluent, 1 mL/min). Dendrimer in Milli-Q solutions were freshly

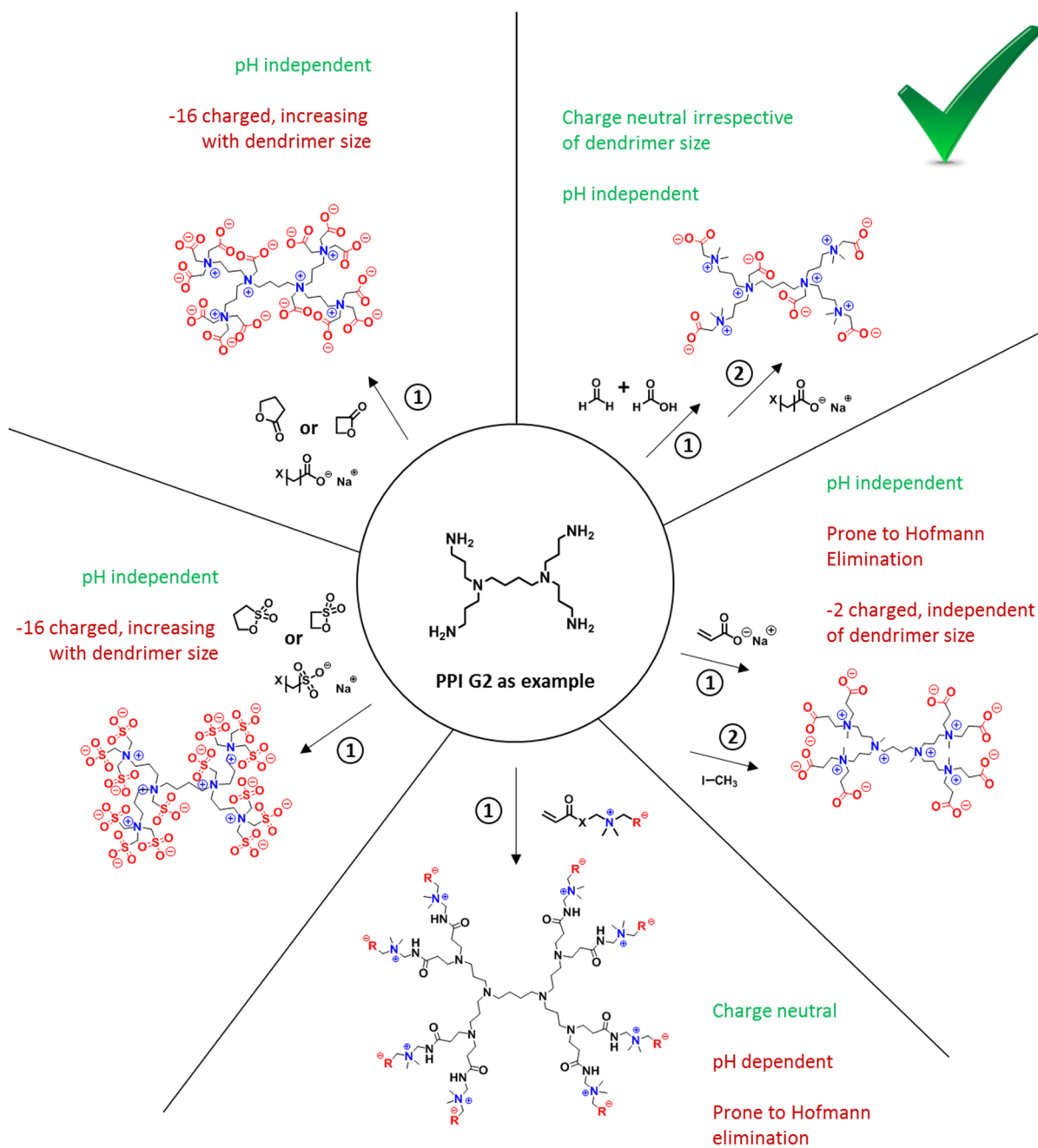
prepared. 20  $\mu$ L was used for each analysis. An Agilent PL2080-0101 PEO calibration kit was used for calibration purposes.

### *Mass Spectrometry (MS)*

MS data were recorded on an Exactive high-resolution MS instrument (Thermo Scientific) equipped with a ESI probe. The MS was calibrated daily using Proteomass LTQ/FT-hybrid ESI Pos. Mode Cal Mix and Pierce ESI Neg. Ion Cal. solutions. Thermo XCalibur Browser software (version 4.0.27.19) was used for instrument control, data acquisition and data processing.

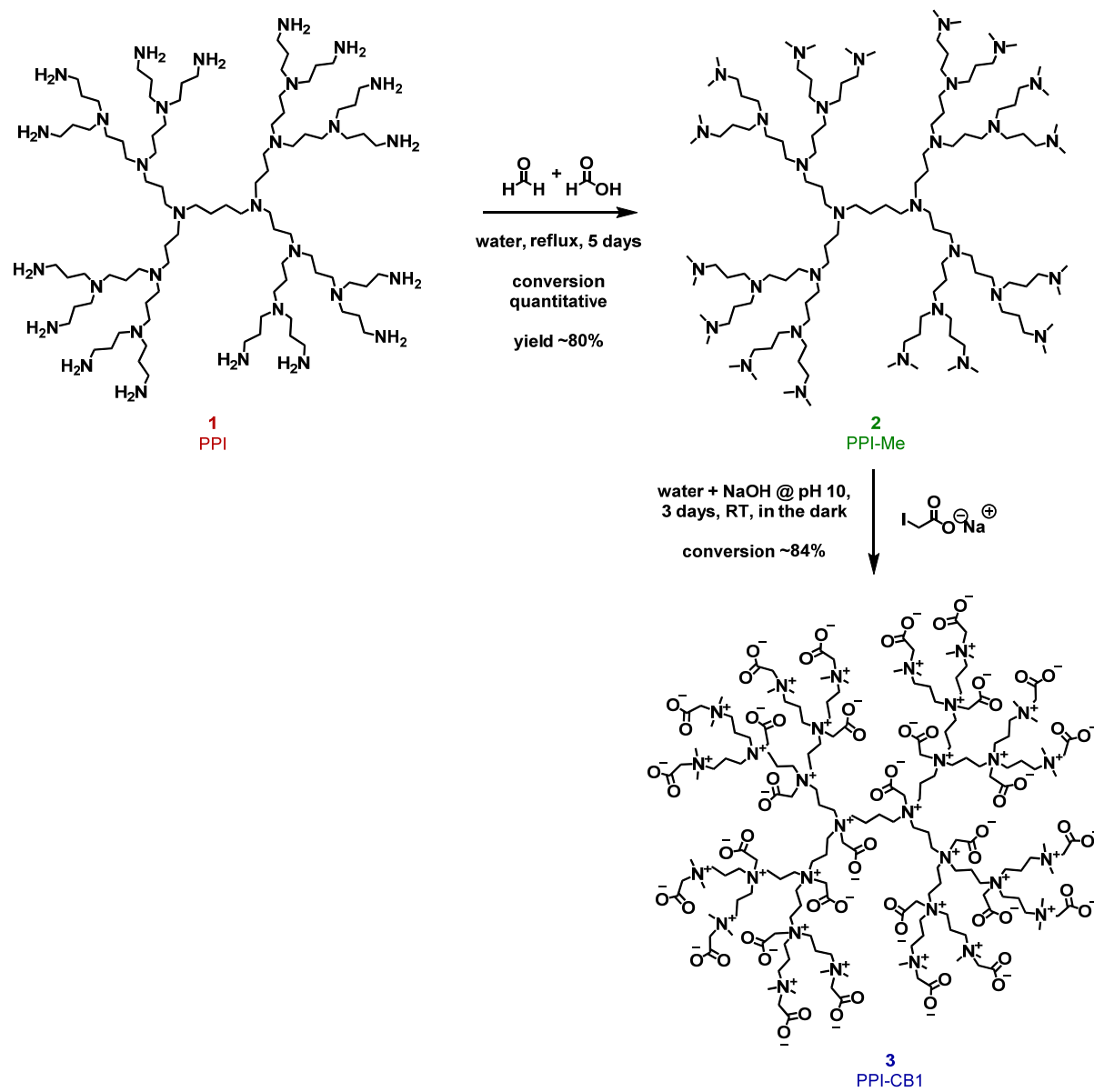
# Synthesis of ZID

## Synthesis of dendrimer 3 PPI-CB1



**Figure S1.** Overview of the various options for the synthesis of zwitterionic dendrimers with their pros (green) and cons (red) with the chosen route highlighted.

Scheme S1. Synthesis of zwitterionic 3 PPI-CB1 dendrimers.



## Synthesis of **2** PPI-Me

A solution of 0.500 g of **1** PPI G3 dendrimer (0.296 mmol) in 10 mL demi water was prepared. A 100 mL 3-neck round bottom flask with a cooler and a stirring bar were flushed with argon by applying 3 vacuum-argon cycles, ending with a final argon refill. Under argon overpressure, 20 mL demi water, 6.35 mL formaldehyde (37% aqueous solution; 75 mmol, 15 eq. per PPI primary amine) and 6.12 mL formic acid (150 mmol, 30 eq. per PPI primary amine) were added. The mixture was cooled on ice before the **1** PPI G3 in 10 mL demi water was added dropwise. The reaction mixture was allowed to warm up to room temperature, after which the setup was closed under argon and refluxed using an oil bath for 5 continuous days to assure full conversion.

Afterwards, the mixture was cooled on ice and the pH was raised to 11 by the slow addition of a saturated NaOH solution. The solution became cloudy since the methylated dendrimers were less water soluble after deprotonation at this concentration. The aqueous solution was extracted with DCM for three times. The combined organic layers were washed with water and dried over Na<sub>2</sub>SO<sub>4</sub>. After evaporation of the solvent, 0.474 g (0.22 mmol) of a yellow oil was obtained with a yield of 80%.

For the synthesis of **2** PPI-Me G2 and G4 the amounts were adjusted in order to retain 15 eq. of formaldehyde and 30 eq. formic acid per PPI primary amine.

**2** PPI-Me characterization:

**<sup>1</sup>H-NMR** (400 MHz, D<sub>2</sub>O, 298K) δ 1.73 (H<sub>1</sub>; s, 4H), δ 1.91 (H<sub>2</sub>; t-overlapping, 56.0H), δ 2.48 (H<sub>3</sub>;s, 96H), δ 2.60, 2.67, 2.75 (H<sub>4</sub>; t-overlapping, 116H) (Figure S5)

**<sup>13</sup>C-NMR** (100 MHz, D<sub>2</sub>O, 298K) δ 23.39, δ 41.45-44.28, δ 51.74, δ 54.94, δ 57.14 see Figure S6

**<sup>13</sup>C-HSQC** (100 MHz, D<sub>2</sub>O, 298K) see Figure S6

**IR** see Figure S188

**XPS** C1s and N1s narrow scans are provided in Figure 2

## Synthesis of **3** PPI-CB1

0.200 g of **2** PPI-Me (0.094 mmol) was dissolved in 3 ml aqueous NaOH solution at pH 10 by stirring in a 10 ml round bottom flask. 2.08 g (10 mmol) sodium iodoacetate was added and the solution was stirred at room temperature in the dark for 3 days. Afterwards, the pH was adjusted to ~7 using an HCl solution to assure compatibility with the dialysis membrane and the volume of the mixture was increased to 10 mL by addition of demi water. The mixture was dialyzed against 500 mL demi water for 3 days with 3 medium exchanges. After evaporation of the solvent and lyophilization, 0.238 g of a fluffy white powder was obtained with a yield of 68%.

For the synthesis of **3** PPI-CB1 G2 and G4 with sodium iodoacetate, the amounts were adjusted in order to retain 4 eq. of sodium iodoacetate per **2** PPI-Me tertiary amine.

**3** PPI-CB1 characterization:

**<sup>1</sup>H-NMR** (400 MHz, D<sub>2</sub>O, 298K) δ 2.29 (H<sub>1</sub>; s, 56.0H), δ 3.27 (H<sub>2</sub>; s-overlapping, 96H), δ 3.71 (H<sub>3</sub>; s, 116H), δ 3.97 (H<sub>4</sub>; s-overlapping, 60H) see Figure S7

**<sup>13</sup>C-NMR** (100 MHz, D<sub>2</sub>O, 298K) (δ 16.45, δ 51.99, δ 56.74-59.80, δ 60.28, δ 61.69, δ 64.00) see Figure S8

**<sup>13</sup>C-HSQC** (100 MHz, D<sub>2</sub>O, 298K) see Figure S8

**DOSY** (400 MHz, D<sub>2</sub>O, 298K) see Figure 5

**COSY** (400 MHz, D<sub>2</sub>O, 298K) see Figure S9

**<sup>1</sup>H-<sup>15</sup>N HMBC** (600 MHz, D<sub>2</sub>O, 298K) see Figure S10

**IR** see Figure S188

**XPS** C1s and N1s narrow scans are provided in Figure 2

**GPC** see Figure S22



### **Optimization Menschutskin alkylation of methylated dendrimers using different alkyl halides**

To optimize the conversion of the alkylation reaction, we tested different dendrimer sizes and alkylation agents and studied the results by XPS N1s high-resolution scans (see Figure S2 and Figure S3).

For the sodium salts *sodium bromoacetate*, *sodium bromoethane sulfonate* and *sodium bromopropane sulfonate* a similar procedure as described for sodium iodoacetate was used, keeping the same equivalents:

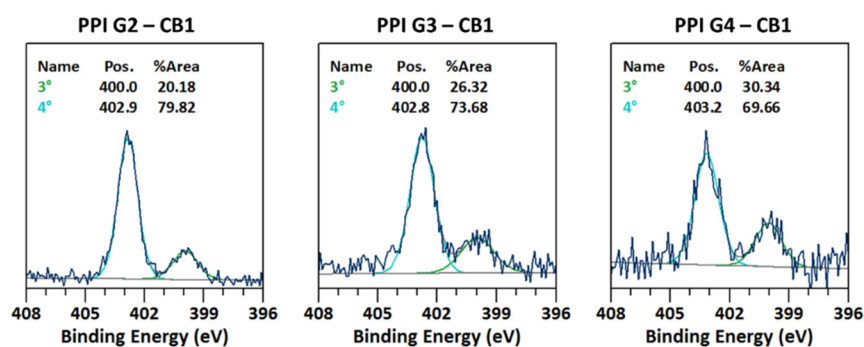
0.200 g of **2** PPI (0.094 mmol) was dissolved in 3 mL aqueous NaOH solution at pH 10 by stirring in a 10 mL round bottom flask. 10 mmol of the alkyl halide sodium salt was added and the solution was stirred at room temperature in the dark for 3 days. Afterwards, the pH was adjusted to ~7 using an aqueous HCl solution to assure compatibility with the dialysis membrane and the volume of the mixture was increased to 10 mL by addition of demi water. The mixture was dialyzed against 500 mL demi water for 3 days with 3 medium exchanges. After evaporation of the solvent and lyophilization, the products were obtained with various yields and conversions (see Figure S3).

For the reaction with protected acids or *methyl iodide* instead of free acetates (*tert-butyl iodoacetate*, *tert-butyl bromoacetate*, *methyl iodoacetate*, *methyl bromoacetate*) an adjusted protocol was followed, using the same equivalents. For solubility reasons, acetonitrile was used as a solvent instead of aqueous NaOH solution. Afterwards, the solvent and other volatiles were evaporated *in vacuo* and a deprotection step was performed before purification. To this end, *tert*-butyl-protected CB1 dendrimers were stirred in 50 mmol TFA in 15 mL acetonitrile (3.3M) at room temperature for 3 days, followed by evaporation of solvent, TFA and *tert*-butanol. After dissolving the product in demi water, the pH was adjusted to ~7 using a NaOH solution before purification by dialysis.

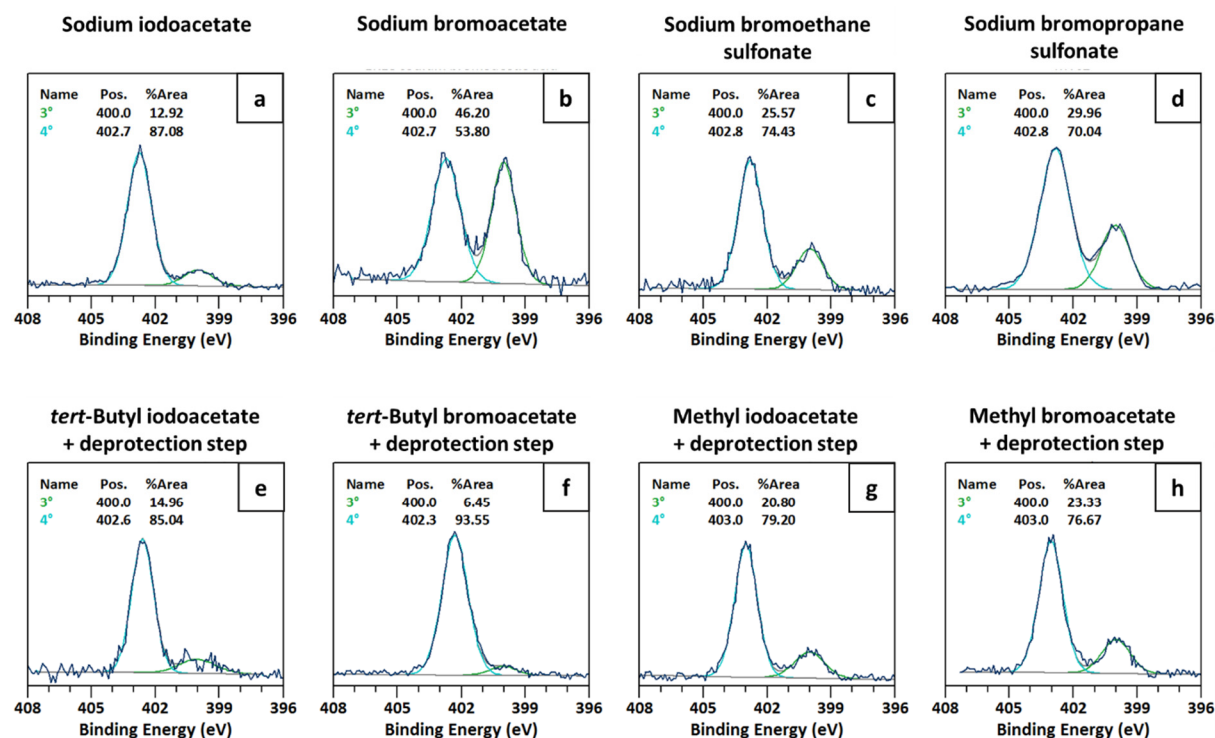
Deprotection of methyl-protected CB1 dendrimers was achieved by refluxing in a pH 10 NaOH solution for 3 days, the pH was adjusted to ~7 using an HCl solution before purification by dialysis. The dendrimer solutions were dialyzed against 500 mL demi water for 3 days with 3 medium exchanges. After evaporation of the solvent and lyophilization, the products were obtained with various conversions (see Table 1 and Figure S3).

## XPS results

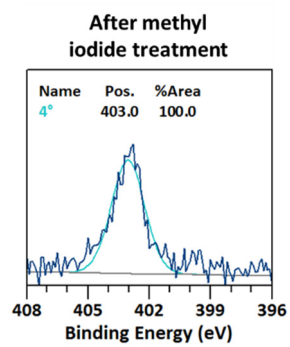
In this section the XPS N 1S wide scan spectra are shown that were obtained during the optimization study to establish the optimal conditions for the second reaction step, during which the tertiary amine groups become quarternized with concomitant installation of negatively charged group (Scheme S1).



**Figure S2.** Effect of dendrimer generation on the conversion of the second reaction step (3° = tertiary amine, 4° = quaternary amine).



**Figure S3.** XPS results for the optimization study of the second reaction step, which was achieved using either the sodium salt (a-d) or the protected acid (e-h) of the indicated alkyl halides (3° = tertiary amine, 4° = quaternary amine).

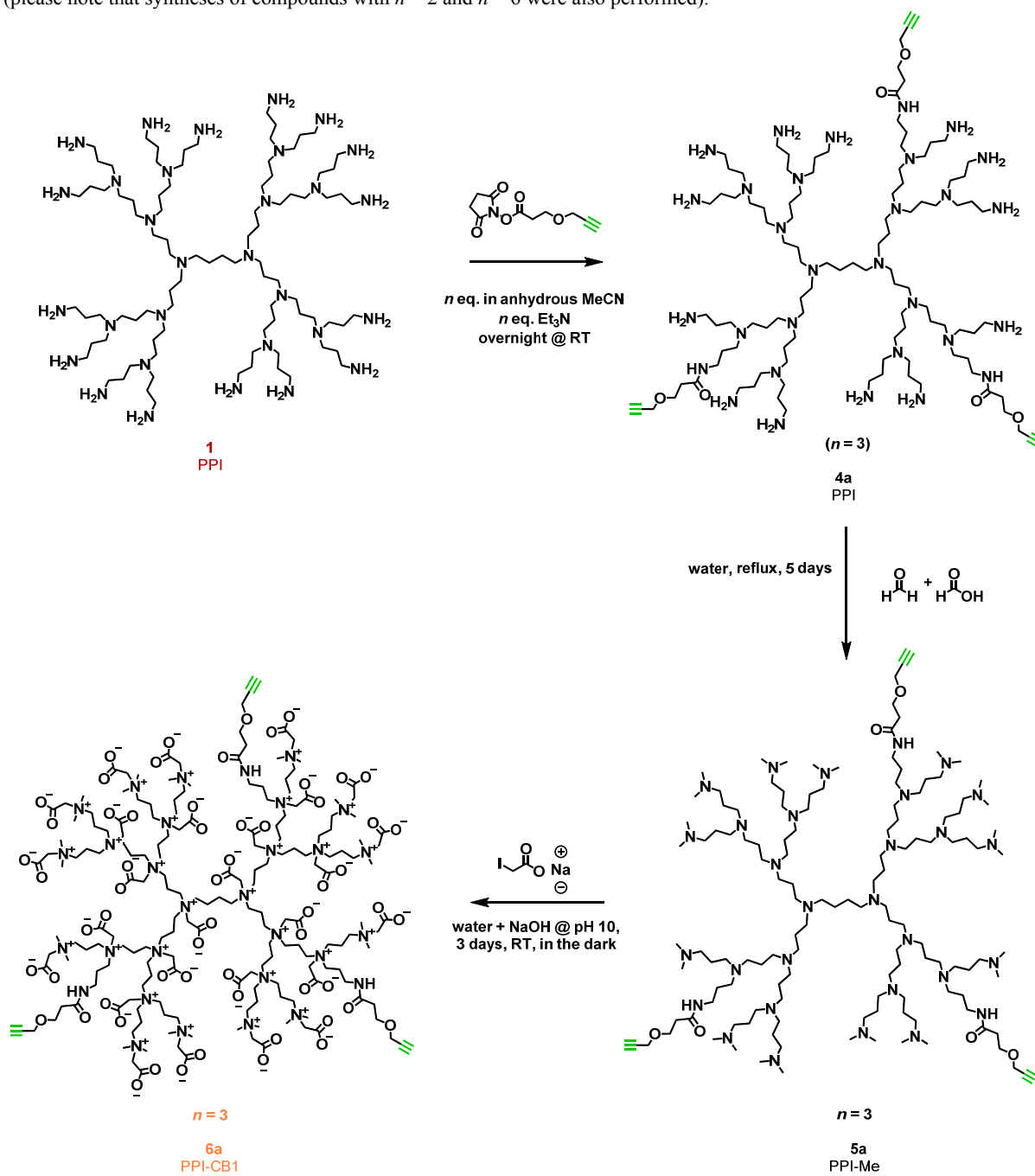


**Figure S4.** XPS results after treatment of an almost 100% quaternary zwitterionic dendrimer with methyl iodide as strong alkylating agent (4° = quaternary amine).

## Synthesis dendrimer 6a

**Scheme S2.** Synthesis of alkyne-functionalized zwitterionic PPI-CB1 dendrimers for  $n = 3$

(please note that syntheses of compounds with  $n = 2$  and  $n = 6$  were also performed).



## Synthesis of alkyne-functionalized PPI dendrimer **4a**

50 mg of **1** PPI G3 (0.029 mmol) was dissolved in 5 mL dry acetonitrile by stirring in an argon flushed 25 mL round bottom flask. Then, 9  $\mu$ L (0.062 mmol,  $n = 2$ ), 13  $\mu$ L (0.093 mmol,  $n = 3$ ), or 26  $\mu$ L (0.186 mmol,  $n = 6$ ) of triethylamine was added and the solution was cooled on ice. A solution of 13.5 mg (0.060 mmol,  $n = 2$ ), 20.3 mg (0.090 mmol,  $n = 3$ ) or 40.5 mg (0.180 mmol,  $n = 6$ ) of alkyne-NHS in 5 mL dry acetonitrile was slowly added to the **1** PPI G3 solution while stirring vigorously to assure well distribution of the functional click handles over the dendrimers. The mixture was allowed to warm up to room temperature and stirring was continued overnight under argon. The solvent and triethylamine were removed by rotavap and oil pump vacuum until a viscous colorless oil was left. 10% of the crude was purified using dialysis as described before for MS analysis purposes. From this fraction, an average yields of ~90% could be calculated. The rest of the crude product was used for the next reaction step without further purification.

## Synthesis of alkyne-functionalized PPI-Me **5a**

The functionalized dendrimer **4a** was methylated as described before for compound **2**, using the same equivalents, conditions and purification by extraction\*. This lead to methylated, functionalized dendrimers with typical yields of ~70%.

\* After extraction there were still some minor impurities present in NMR, which are fully remove after extensive dialysis upon the next modification step.

## Synthesis of alkyne-functionalized PPI-CB1 **6a**

The methylated, functionalized dendrimers were alkylated using sodium iodoacetate as described previously for compound **3**. This yielded alkyne modified ZID with typical yields of 30% (not taking into account that the conversion in the last step is not quantitative).

**6a** PPI-CB1 characterization:

**<sup>1</sup>H-NMR** (400 MHz, D<sub>2</sub>O, 300 K) δ 2.29 (H<sub>1</sub>; m, 50H), δ 2.56 (H<sub>2</sub>; m, 6H), δ 2.95 (H<sub>3</sub>; t, 6H), δ 3.28 (H<sub>4</sub>; s, 78H), δ 3.33 (H<sub>5</sub>; s, 3H), δ 3.39 (H<sub>6</sub>; t, 6H), δ 3.70 (H<sub>7</sub>; t, 110H), δ 3.95 (H<sub>8</sub>; s-overlapping, 54H), δ 4.04 (H<sub>9</sub>; t-overlapping, 6H), δ 4.23 (H<sub>10</sub>; s, 6H) (Figure S11)

**<sup>13</sup>C-NMR** see Figure S12

**DEPT-HSQC** see Figure S12

**IR** see Figure S199

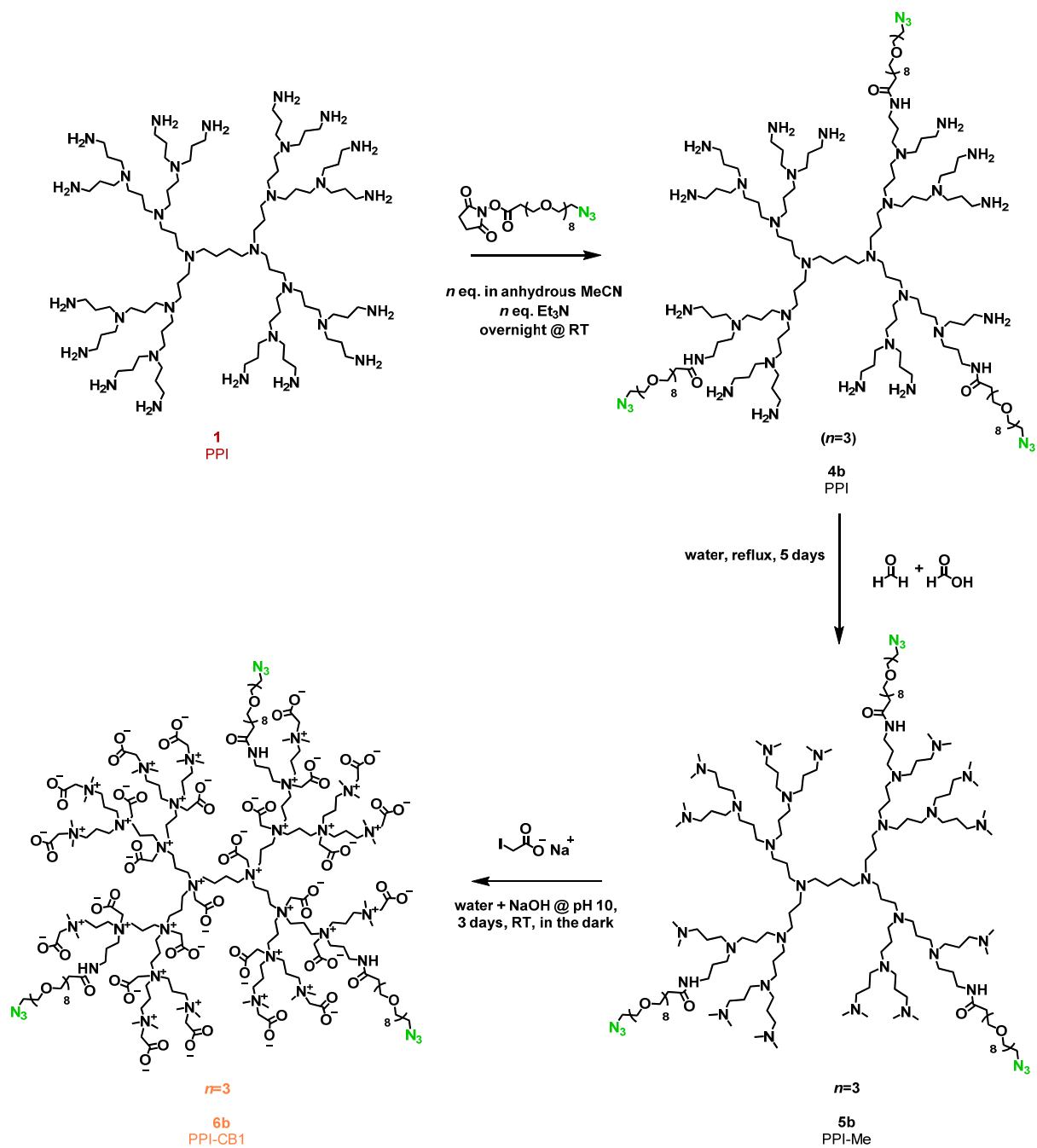
**XPS** C1s and N1s narrow scans are provided in Figure 3

**MS** see Figure S20 and Figure S21

**GPC** see Figure S23 and Figure S24

## Synthesis dendrimer 6b

**Scheme S3.** Synthesis of azido-functionalized zwitterionic PPI-CB1 dendrimers for  $n = 3$  (please note that syntheses of compounds with  $n = 6$  was also performed).



## Synthesis of azide-functionalized PPI dendrimer **4b**

50 mg of **1** PPI G3 (0.029 mmol) was dissolved in 5 mL dry acetonitrile by stirring in an argon flushed 25 mL round bottom flask. Then, 13  $\mu$ L (0.093 mmol,  $n = 3$ ) or 26  $\mu$ L (0.186 mmol,  $n = 6$ ) of triethylamine was added and the solution was cooled on ice. A solution of 50.8 mg (0.090 mmol,  $n = 3$ ) or 101.6 mg (0.180 mmol,  $n = 6$ ) of azide-PEG-NHS in 5 mL dry acetonitrile was slowly added to the **1** PPI G3 solution while stirring vigorously to assure well distribution of the functional handles over the dendrimers. The mixture was allowed to warm up to room temperature and stirring was continued overnight under argon. The solvent and triethylamine were removed by rotavap and oil pump vacuum until a viscous colorless oil was left. The crude product was used for the next reaction step without further purification.

## Synthesis of azide-functionalized PPI-Me **5b**

The functionalized dendrimer **4b** was methylated as described before for compound **2**, using the same equivalents, conditions and purification by extraction\*. This lead to methylated, functionalized dendrimers with an average yield of ~89%.

\* After extraction there were still some minor impurities present in NMR, which are fully remove after extensive dialysis upon the next modification step.

## Synthesis of azide-functionalized PPI-CB1 **6b**

The methylated, functionalized dendrimers were alkylated using sodium iodoacetate as described previously for compound **3**. This yielded alkyne modified ZID with an average yield of ~68%.

**6b** PPI-CB1 characterization:

**<sup>1</sup>H-NMR** (400 MHz, D<sub>2</sub>O, 300 K)  $\delta$  2.27 (H<sub>1</sub>; m, 50H),  $\delta$  2.54 (H<sub>2</sub>; s, 6H),  $\delta$  2.92 (H<sub>3</sub>; m, 6H),  $\delta$  3.26 (H<sub>4</sub>; t, 78H),  $\delta$  3.37 (H<sub>5</sub>; s, 6H),  $\delta$  3.69 (H<sub>6</sub>; t, 108H),  $\delta$  3.77 +  $\delta$  3.84 (H<sub>7+8</sub>; t-overlapping, 113H),  $\delta$  3.93 (H<sub>9</sub>; d, 54H) (Figure S13)

**<sup>13</sup>C-NMR** see Figure S14

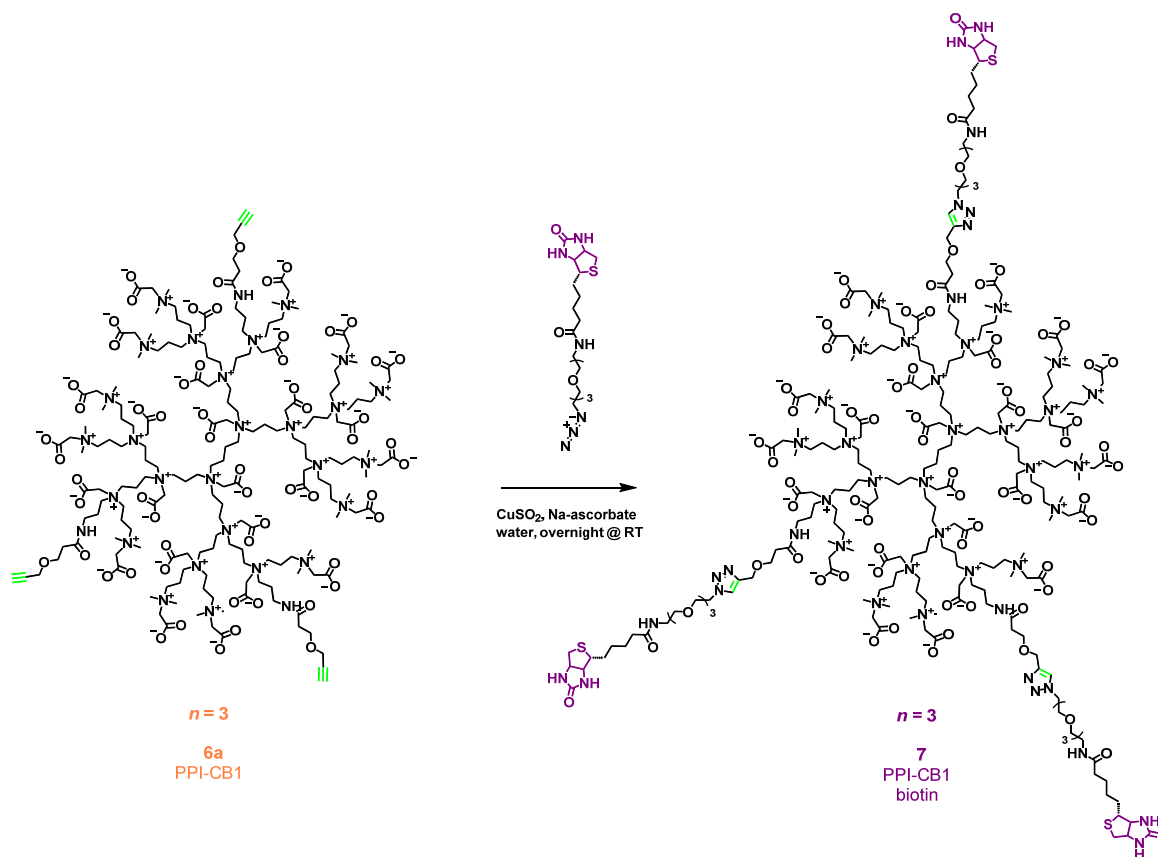
**DEPT-HSQC** see Figure S14

**IR** see Figure S199



## Synthesis of biotin-functionalized PPI dendrimer 7

**Scheme S4.** Synthesis of biotin-functionalized zwitterionic PPI-CB1 dendrimers for  $n = 3$



A solution of 21.6 mg (0.005 mmol) alkyne functionalized ZID **6a** ( $n = 3$ ) in 1.6 ml milli-Q water was prepared. To this solution, 0.2 ml of a 1 mg/ml solution (0.001 mmol) of copper(II) sulfate pentahydrate in milli-Q water was mixed with 0.2 ml of a 200 mg/ml solution (0.2 mmol) of sodium ascorbate in milli-Q water. This mixture was added to the dendrimer **6a** solution after which 10.8 mg (0.024 mmol) azide-PEG3-biotin was added. The solution was stirred overnight at room temperature. The mixture was dialyzed (MWCO 1000-5000 Da) against 500 mL demi water for 3 days with 3 medium exchanges. After evaporation of the solvent and lyophilization, 20.6 mg of a fluffy light yellow powder was obtained.

7 PPI-CB1  $n = 3$  characterization:

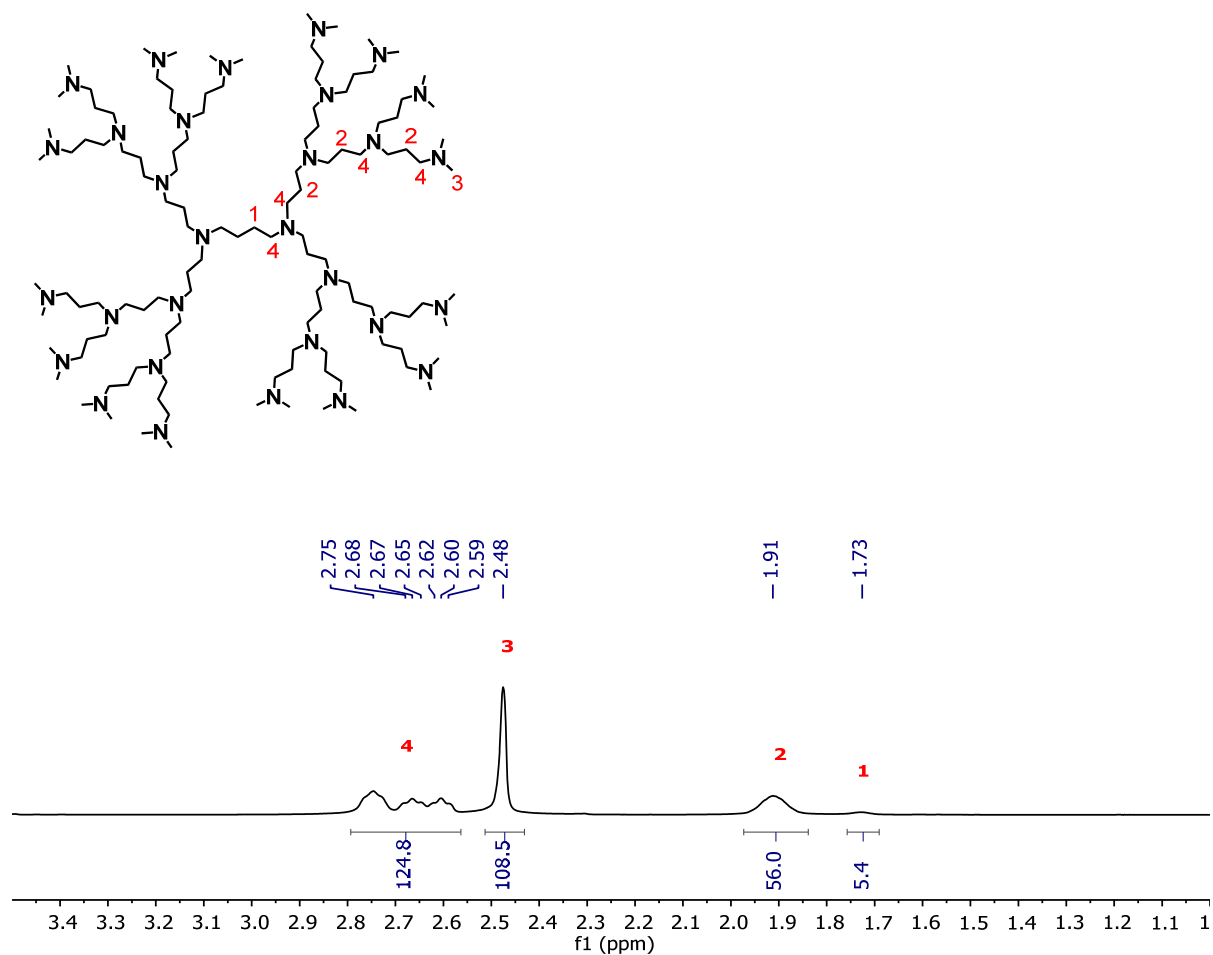
$^1\text{H-NMR}$  see Figure S15

$^{13}\text{C-NMR}$  see Figure S16

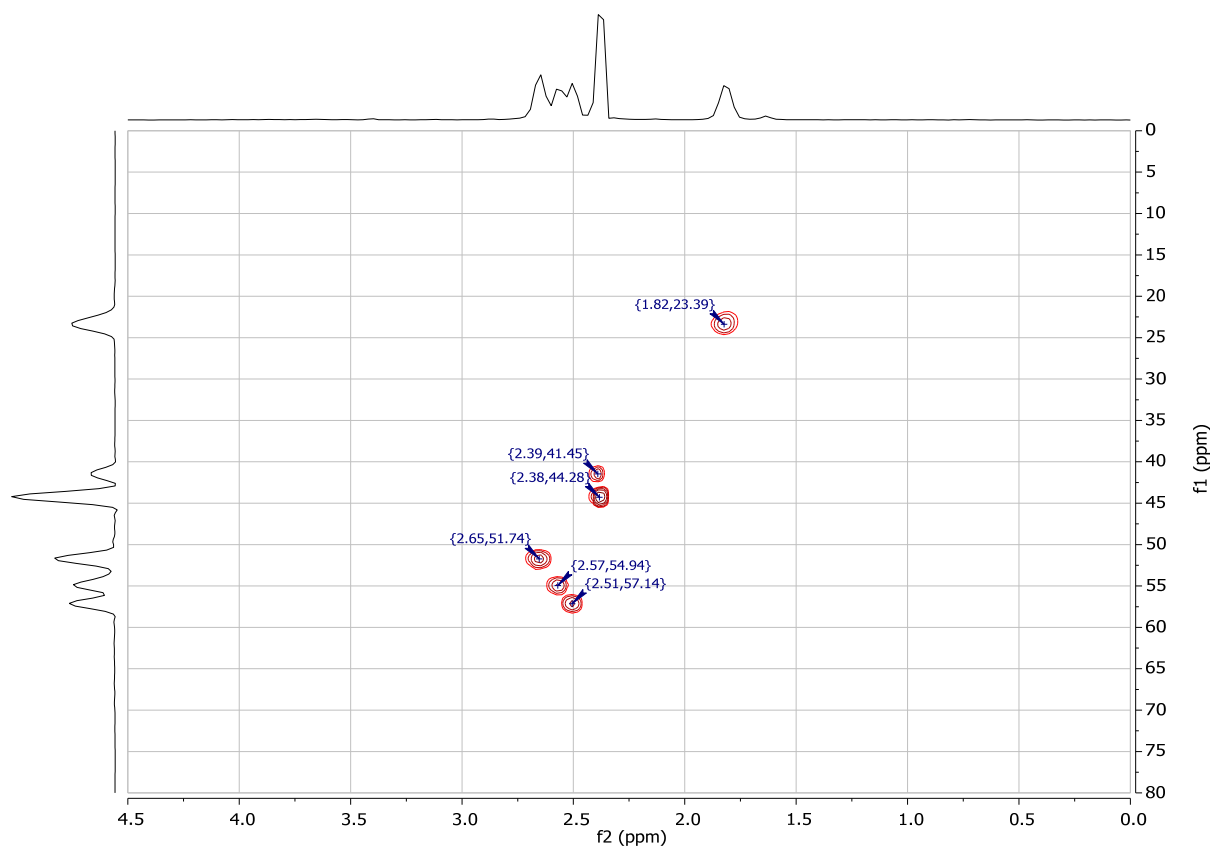
$^{13}\text{C-HSQC}$  see Figure S16

# NMR data and calculations

## Dendrimer **2** PPI-Me

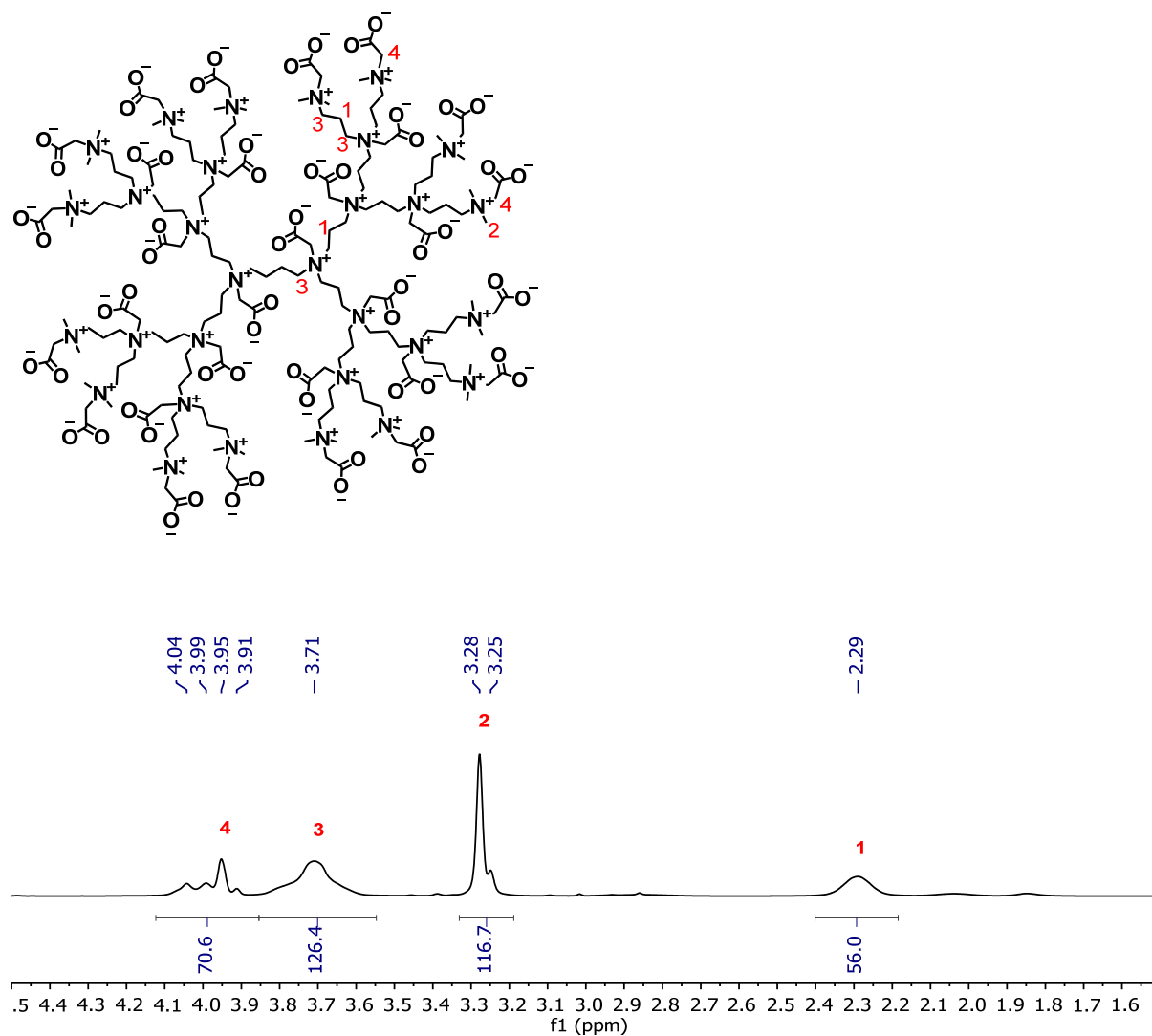


**Figure S5.** <sup>1</sup>H-NMR spectrum of **2** PPI-Me in D<sub>2</sub>O (400 MHz, 298 K).

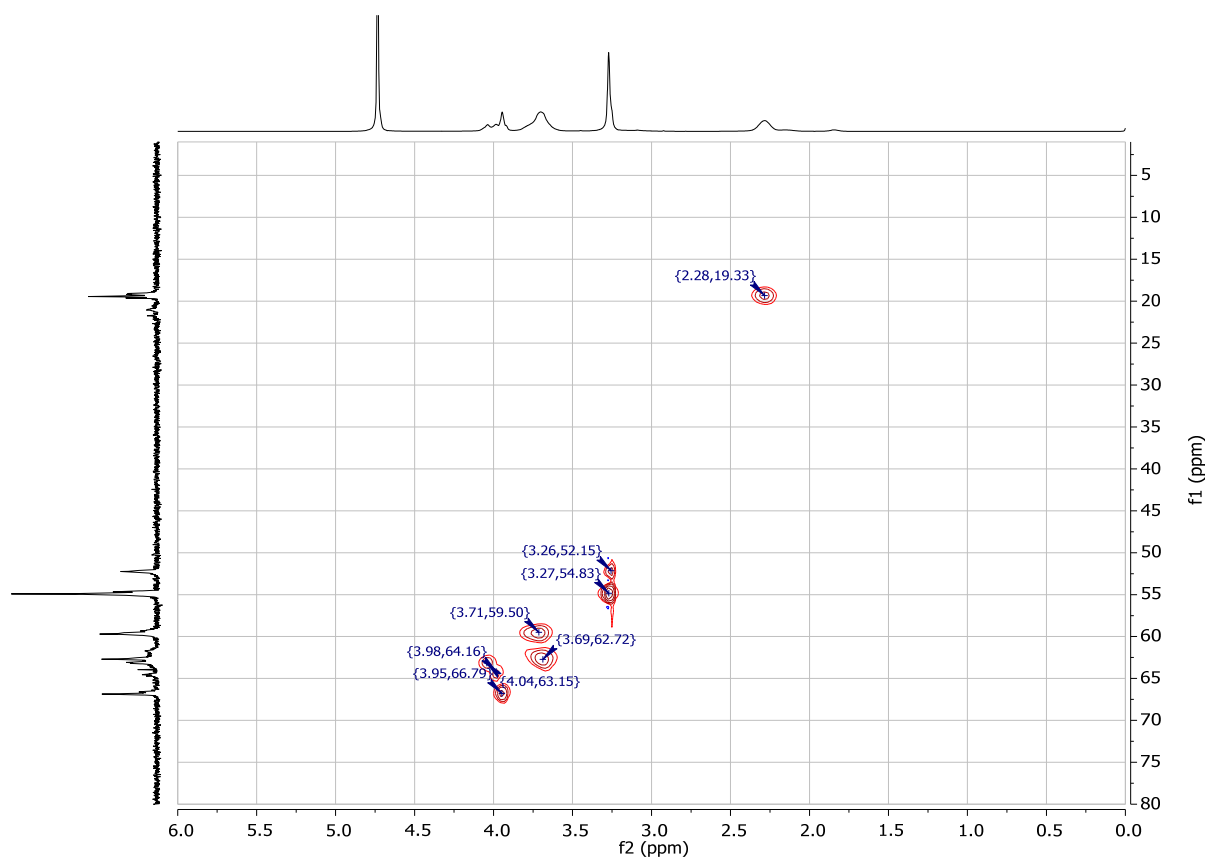


**Figure S6.** HSQC-NMR spectrum of **2** PPI-Me in D<sub>2</sub>O (400 MHz, 298 K). The split up of peaks at 2.39,41.45 and 2.38,44.28 is caused by methyl peaks that are coupled to originally secondary amines (due to minor defects in the starting material **1** PPI<sup>S1</sup> or rearrangements during the methylation<sup>S2</sup>).

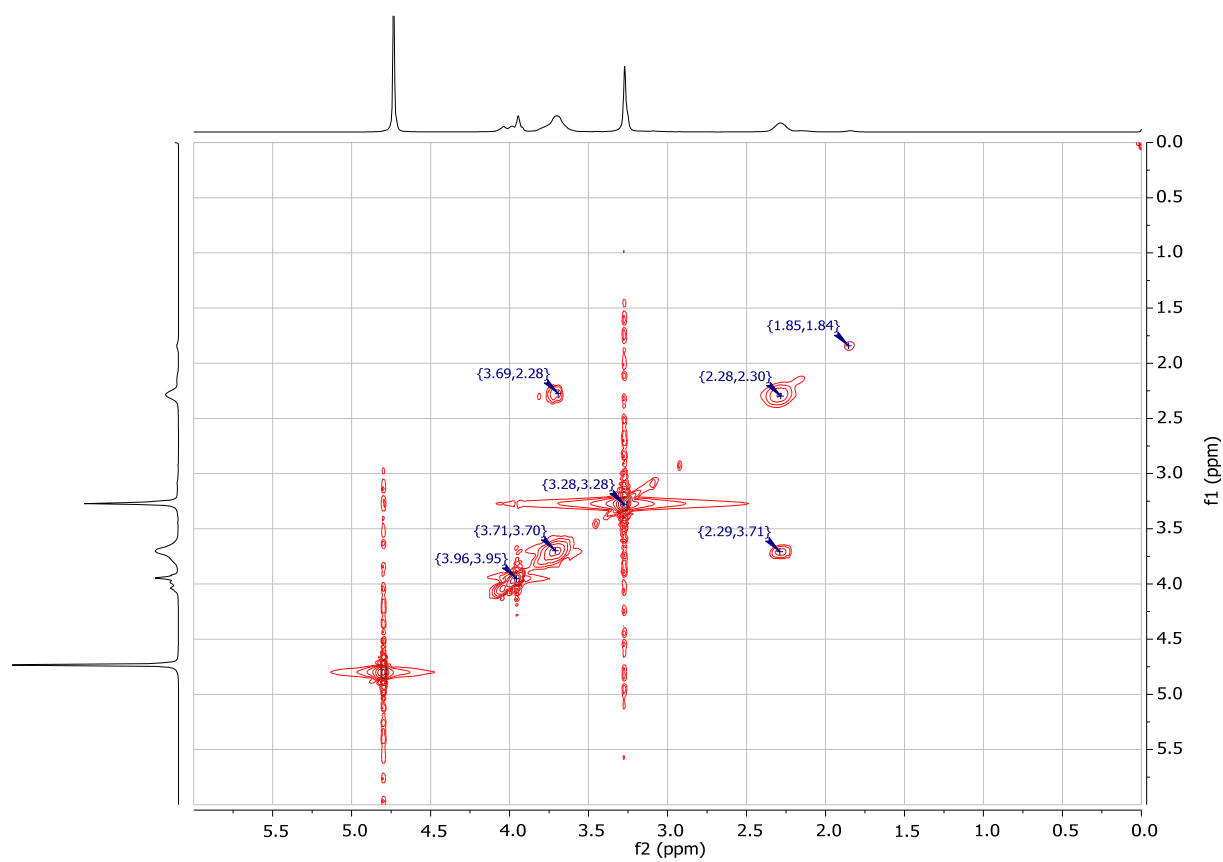
## Dendrimer 3 PPI-CB1



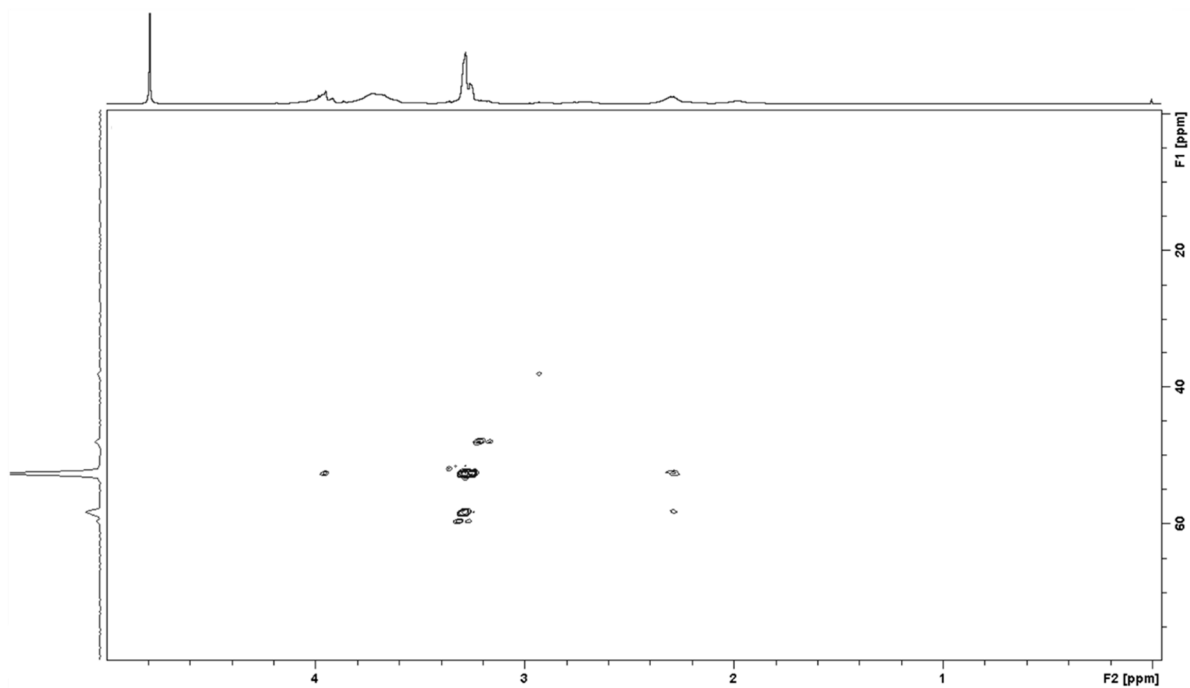
**Figure S7.** <sup>1</sup>H-NMR spectrum of **3** PPI-CB1 in D<sub>2</sub>O (400 MHz, 298 K). The split up of peaks at 3.25 and 3.28 is caused by methyl peaks that are coupled to originally secondary amines (minor defects in the starting material **1** PPI). Peak H<sub>4</sub> is split up in multiple peaks because of the various shells in the dendrimer.



**Figure S8.** HSQC spectrum of **3** PPI-CB1 in D<sub>2</sub>O (400 MHz, 298 K).

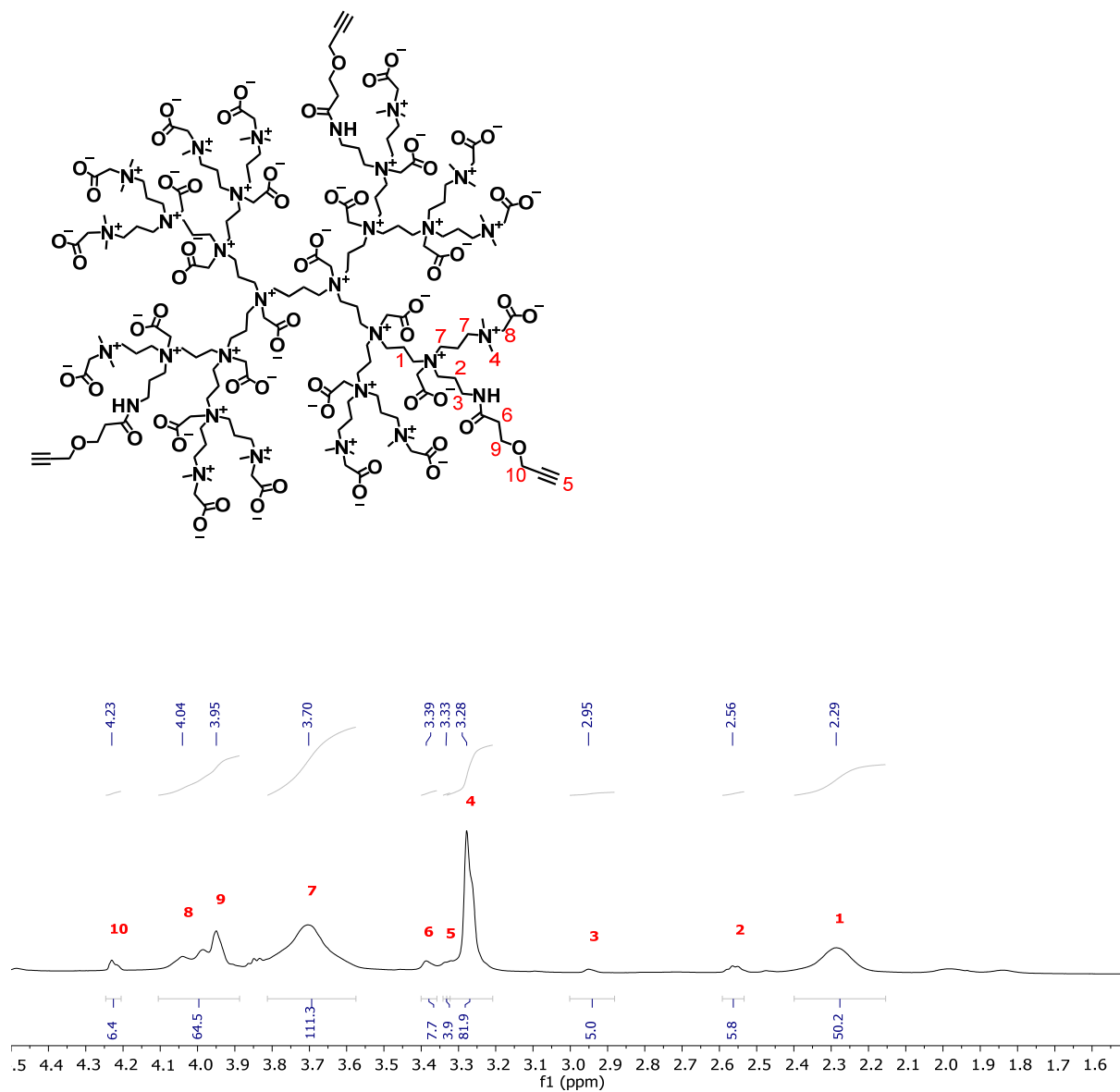


**Figure S9.** COSY spectrum of **3** PPI-CB1 in D<sub>2</sub>O (400 MHz, 298 K).



**Figure S10.**  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of **3** PPI-CB1 in  $\text{D}_2\text{O}$  (600 MHz, 298 K). On the vertical axis, a projection of the 2D-spectrum is shown.

## Dendrimer **6a** ( $n = 3$ ) PPI-CB1



**Figure S11.**  $^1\text{H-NMR}$  spectrum of **6a** ( $n = 3$ ) PPI-CB1 in  $\text{D}_2\text{O}$  (400 MHz, 298 K). The spectrum was normalized by setting the combined integrals of peaks labelled 1 and 2 to a value of 56. For quantification of  $n$ , integrals of peak 3 and 10 (both free protons originating from a  $\text{CH}_2$  group present in the alkyne linker) were considered. Based on their average integral value of 5.7 an average value for  $n$  was calculated to be 2.9.

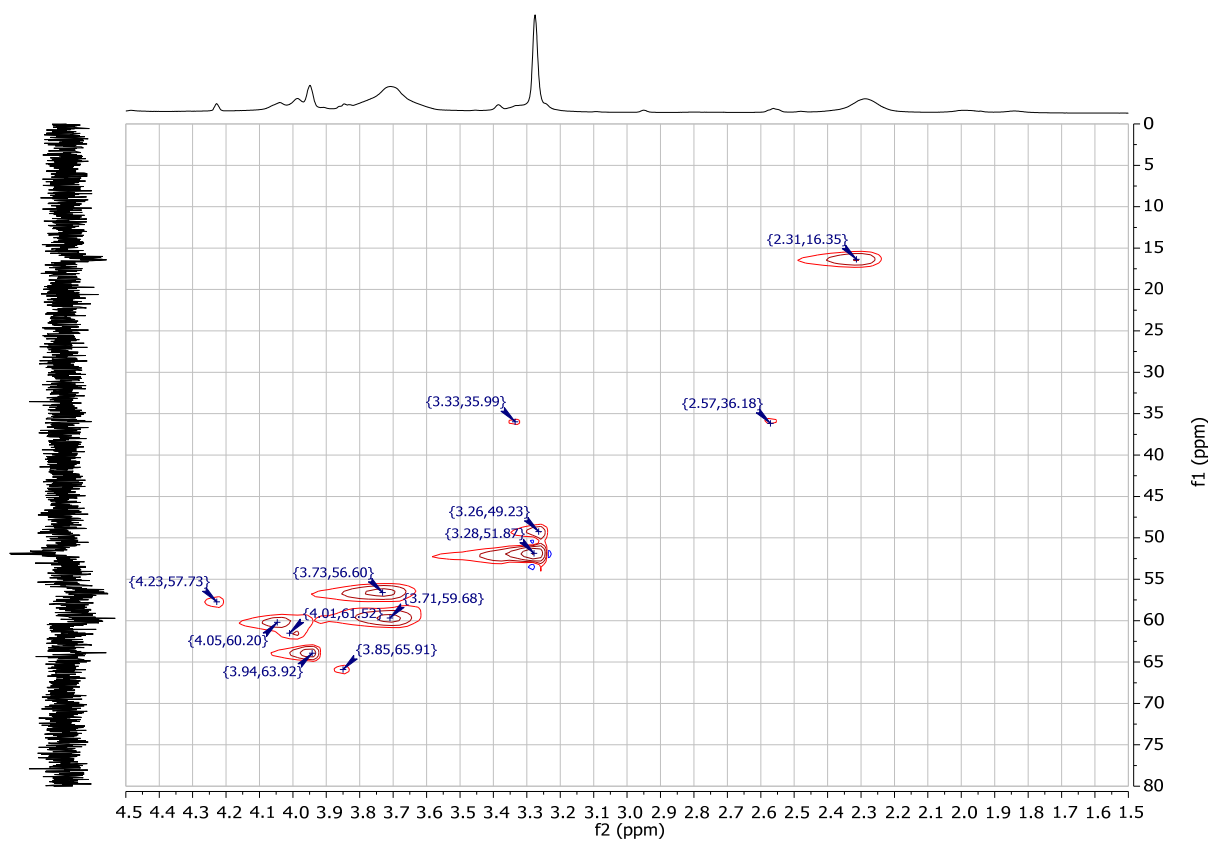
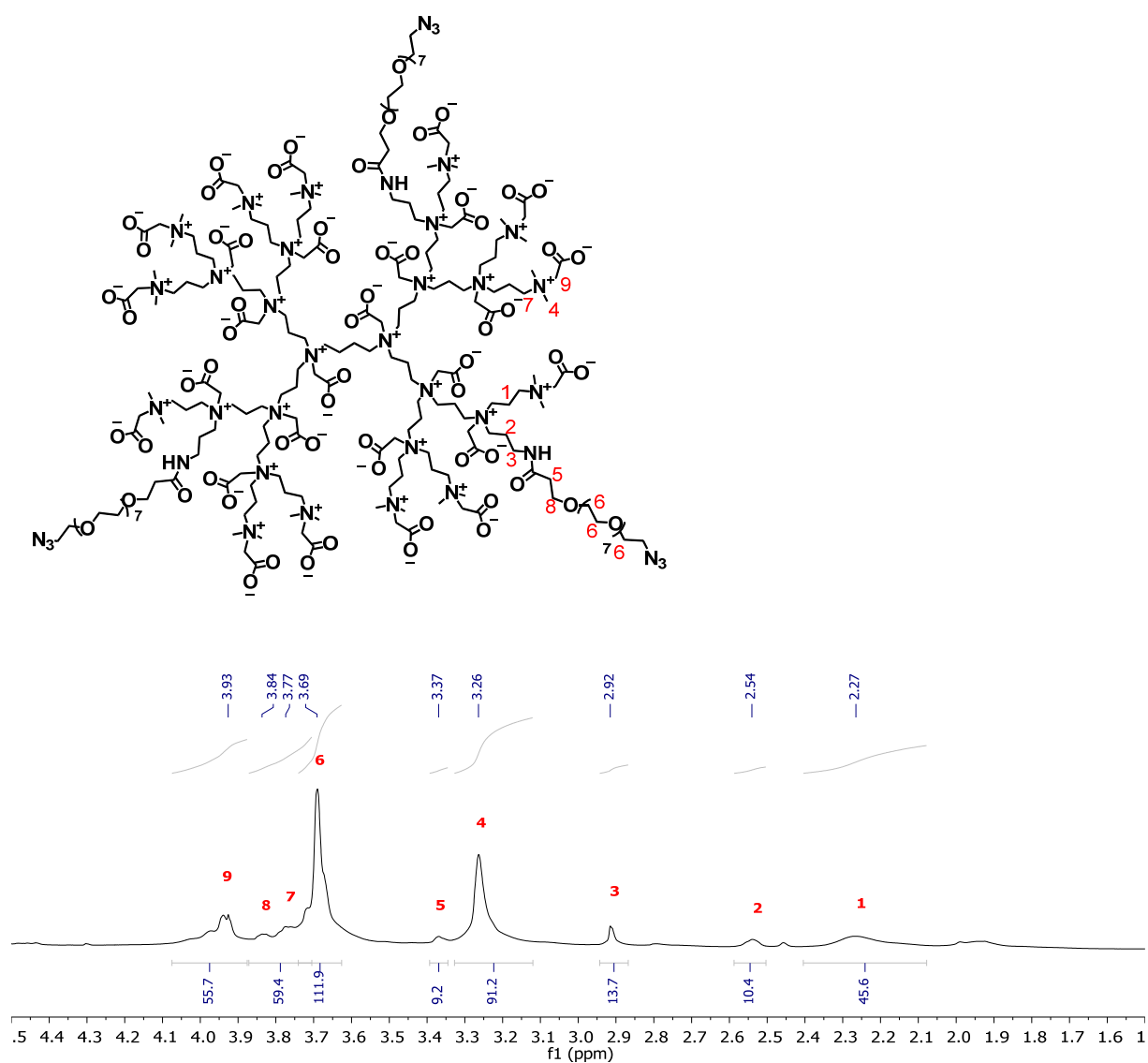


Figure S12. DEPT-HSQC spectrum of **6a** ( $n = 3$ ) PPI-CB1 in D<sub>2</sub>O (400 MHz, 298 K).



# Dendrimer **6b** ( $n = 3$ ) PPI-CB1



**Figure S13.**  $^1\text{H-NMR}$  **6b** ( $n = 3$ ) PPI-CB1 (400 MHz, 298 K).

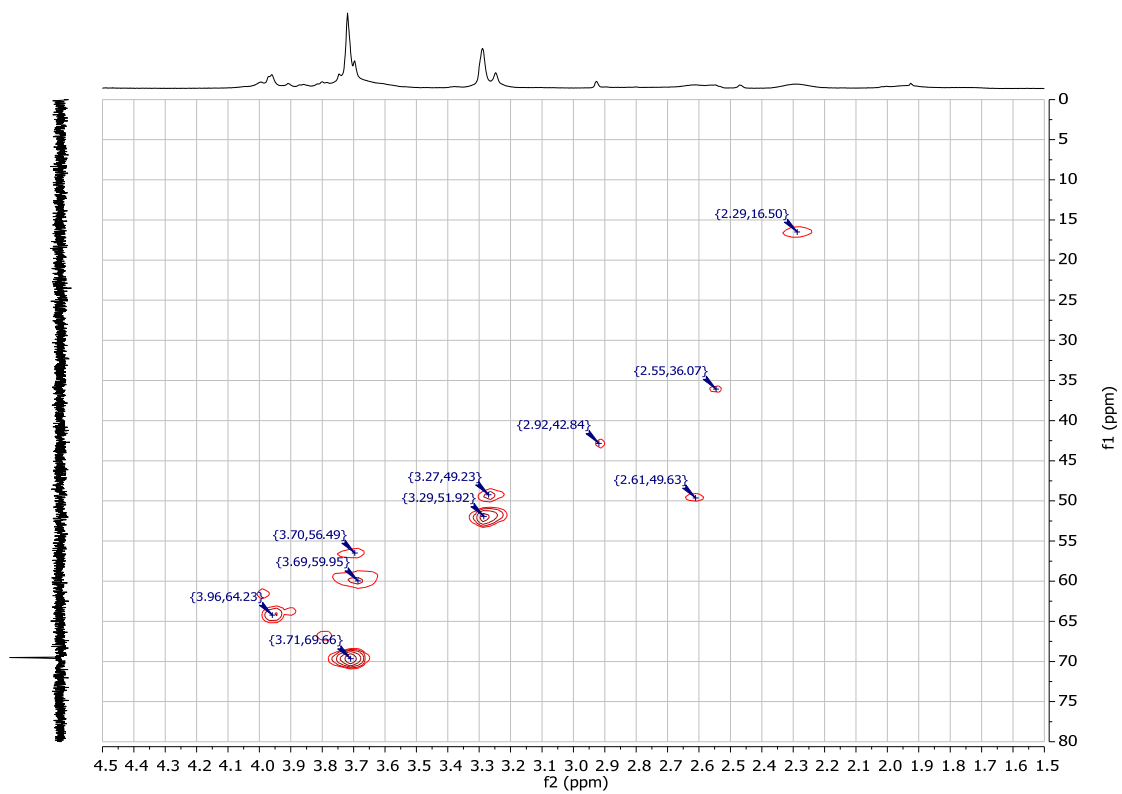
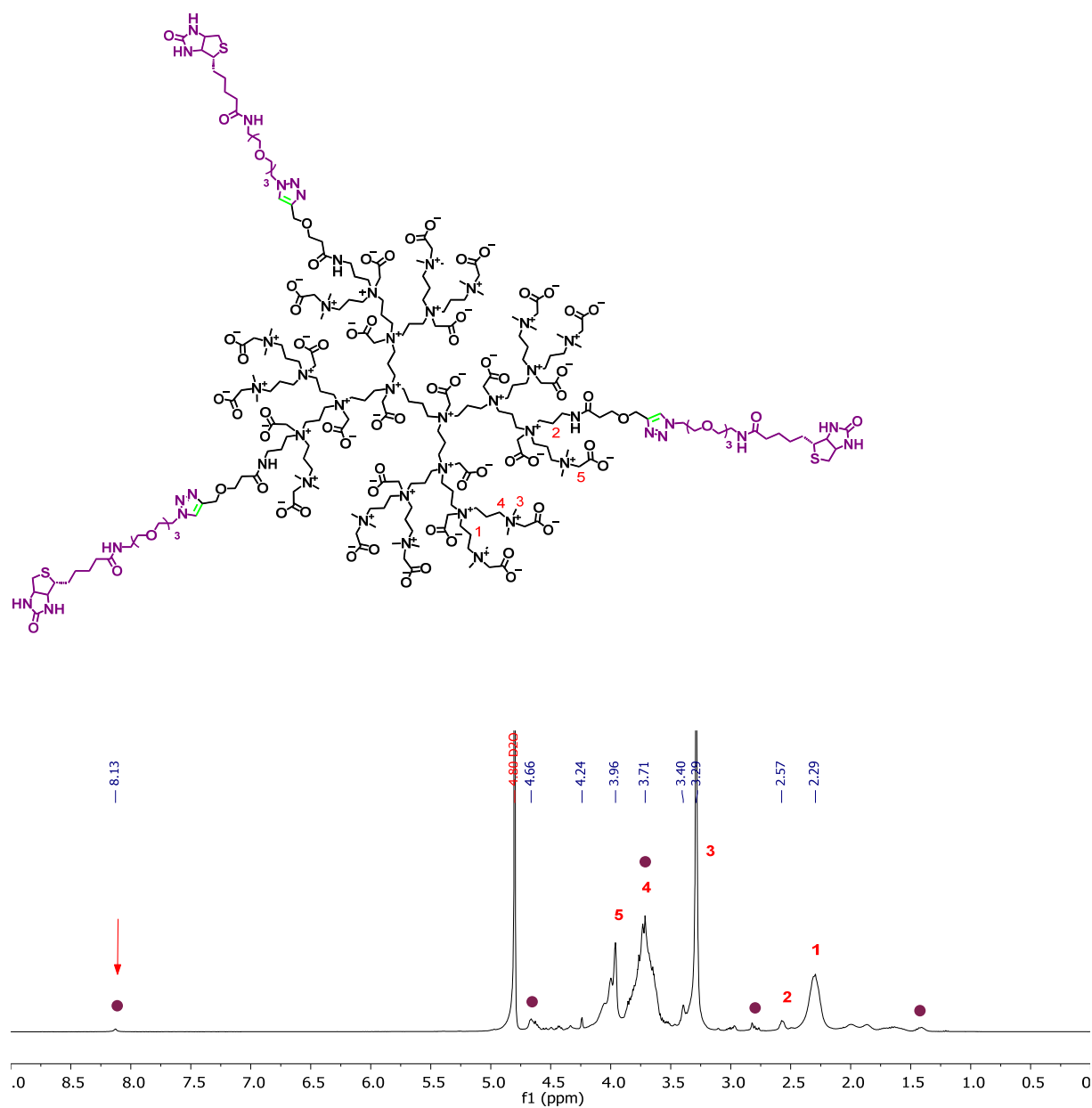
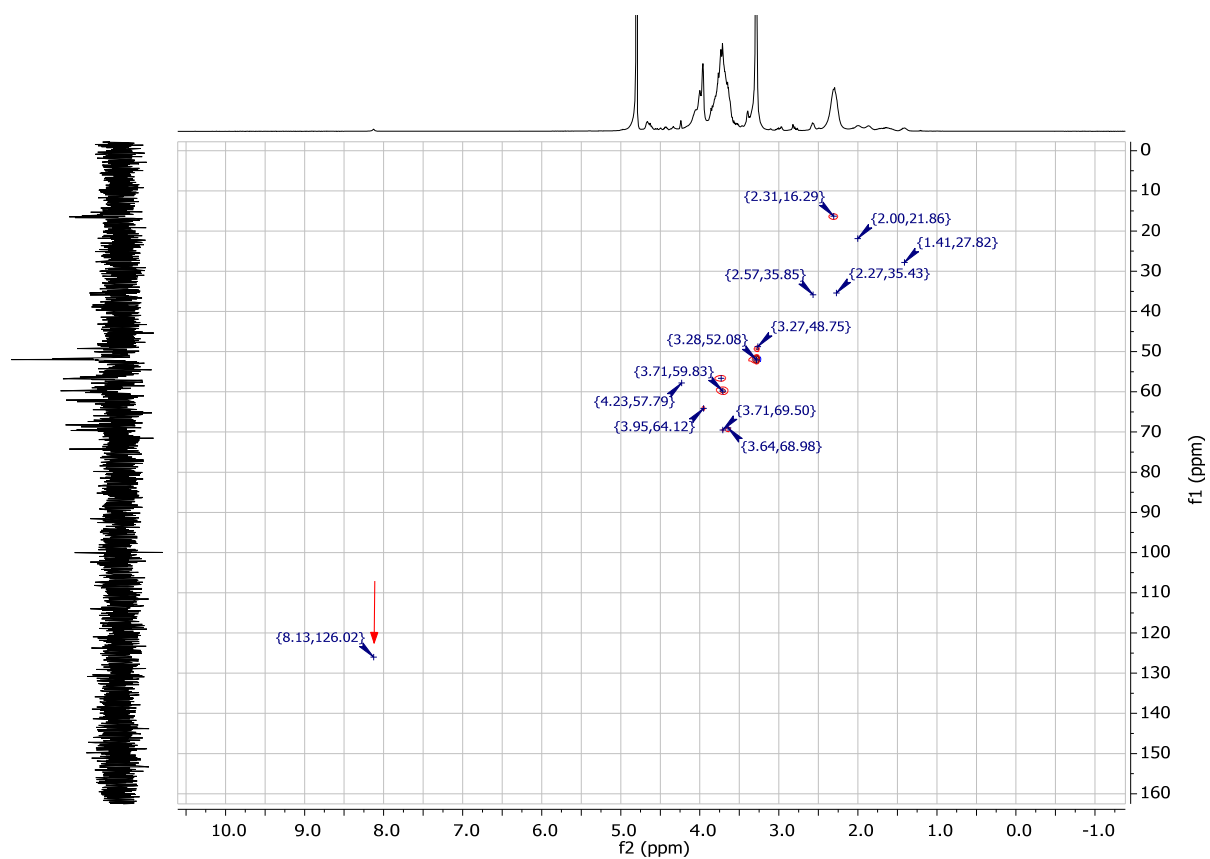


Figure S14. DEPT-HSQC spectrum of **6b** ( $n = 3$ ) PPI-CB1 in D<sub>2</sub>O (400 MHz, 298 K).

## Dendrimer 7 ( $n = 3$ ) PPI-CB1 biotin

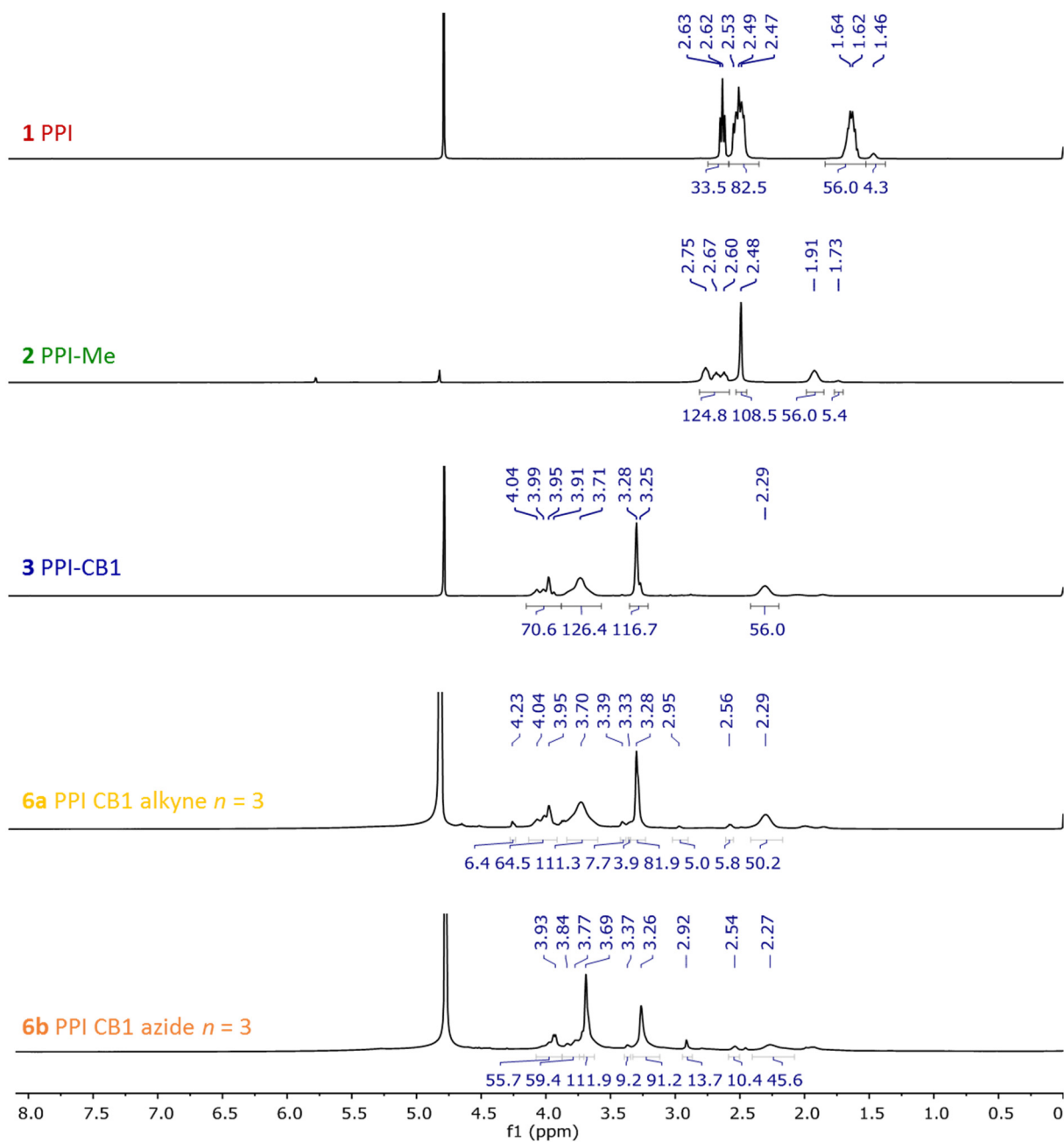


**Figure S15.**  $^1\text{H}$  NMR spectrum of 7 ( $n = 3$ ) PPI-CB1 biotin in  $\text{D}_2\text{O}$  (400 MHz, 298 K). Most important dendrimer backbone signals are indicated in red and signals coming from the biotin and the PEG linker are indicated in purple. The most indicative peak is assigned with a red arrow; the formed triazole (8.13 ppm).



**Figure S16.** <sup>13</sup>C-HSQC spectrum of **7** ( $n = 3$ ) PPI-CB1 biotin in D<sub>2</sub>O (400 MHz, 298 K). The most indicative peak is assigned with a red arrow which originates from the formed triazole (8.13, 126.02 ppm), confirming the desired click reaction has taken place.

# Stacked plot of $^1\text{H}$ -NMR spectra



**Figure S17.**  $^1\text{H}$  NMR spectrum of **1**, **2**, **3**, **6a** and **6b** in  $\text{D}_2\text{O}$  (400 MHz, 298 K). Integrals are normalized to a value of 56.0 for the central methylene protons in the repeated propylene chains for all spectra. The peak at 4.8 ppm is  $\text{H}_2\text{O}$ .

## DOSY hydrodynamic radius calculation

Under the assumption that the zwitterionic dendrimers can be regarded as spherical objects, the hydrodynamic radius,  $r_H$ , of this sphere can be calculated from the measured diffusion coefficient,  $D$ , using the Stokes-Einstein equation (#S1):<sup>S3,S4,S5</sup>

$$D = \frac{k_B \cdot T}{6\pi \cdot \eta \cdot r_H} \quad (\text{eq. S1})$$

where  $D$  is the diffusion coefficient,  $k_B$  the Boltzmann constant,  $T$  the absolute temperature,  $\eta$  the solvent viscosity, and  $r_H$  the hydrodynamic radius of the molecule.

Table S1 lists the calculated hydrodynamic radius for zwitterionic dendrimers **3**, **6a** and **6b**, determined from the diffusion coefficient as obtained in the DOSY measurement (as shown in Figure 5 of the main text).

**Table S1.** Calculated hydrodynamic diameter based on average diffusion coefficients  $D$  (m<sup>2</sup>/s) measured for ZID **3**, **6a** ( $n = 3$ ) and **6b** ( $n = 3$ ) in D<sub>2</sub>O at 300 K.

ZID	Average $D_{\text{ZID}}$ (m <sup>2</sup> /s)	Hydrodynamic diameter (nm)
<b>3</b>	$1.67 \times 10^{-10}$	3.2
<b>6a</b> ( $n = 3$ )	$1.27 \times 10^{-10}$	4.1
<b>6b</b> ( $n = 3$ )	$1.03 \times 10^{-10}$	5.1

## IR spectroscopy data

Dendrimer **1** PPI, **2** PPI-Me, **3** PPI-CB1

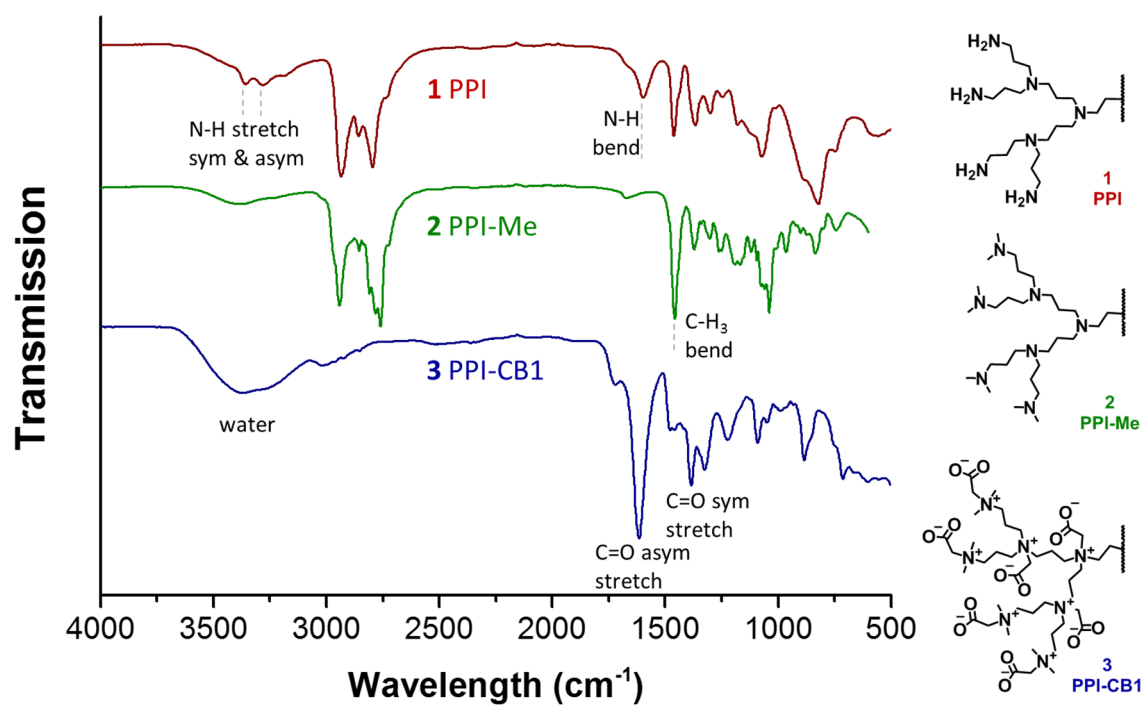


Figure S18. IR spectra of **1**, **2** and **3**. For clarity, on the  $y$ -axis the spectra are shown with an offset with respect to each other.

Dendrimer **6a** ( $n = 6$ ) PPI-CB1, **6b** ( $n = 6$ ) PPI-CB1

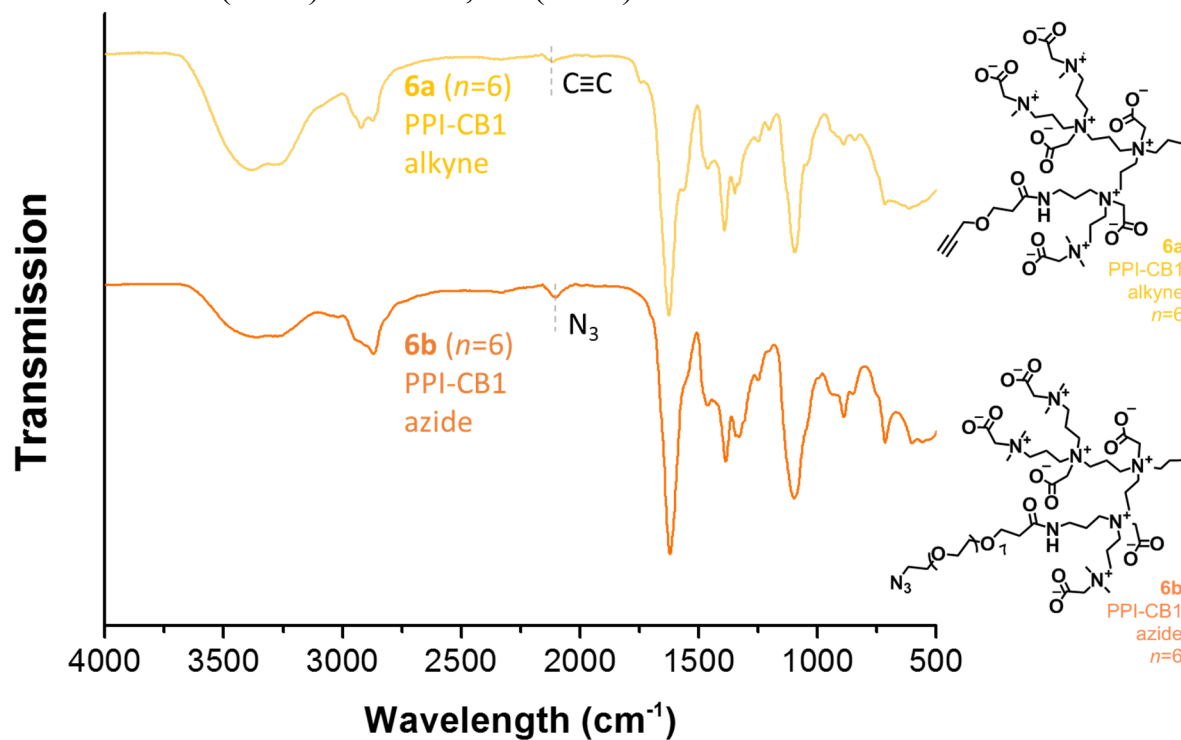
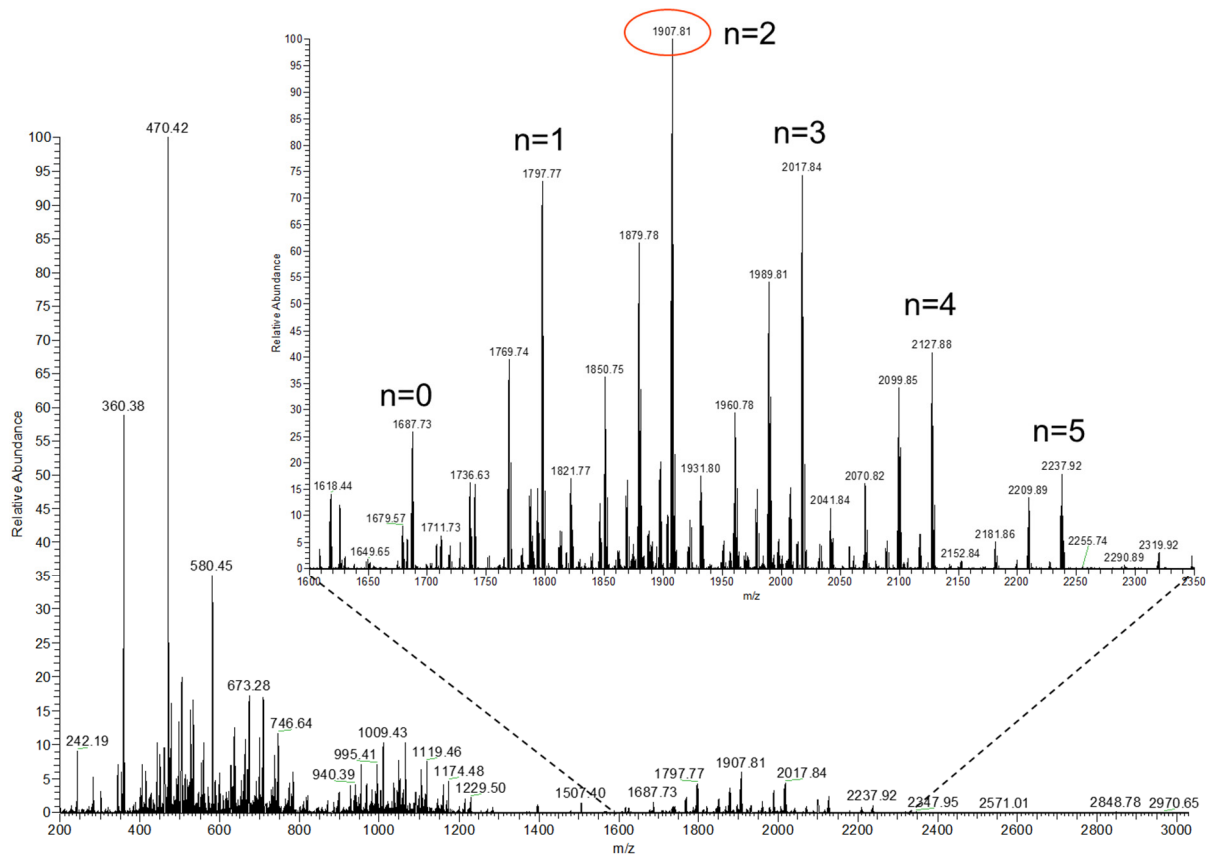


Figure S19. IR spectra of **6a** and **6b**. For clarity, on the  $y$ -axis the spectra are shown with an offset with respect to each other.

# Mass Spectrometry data

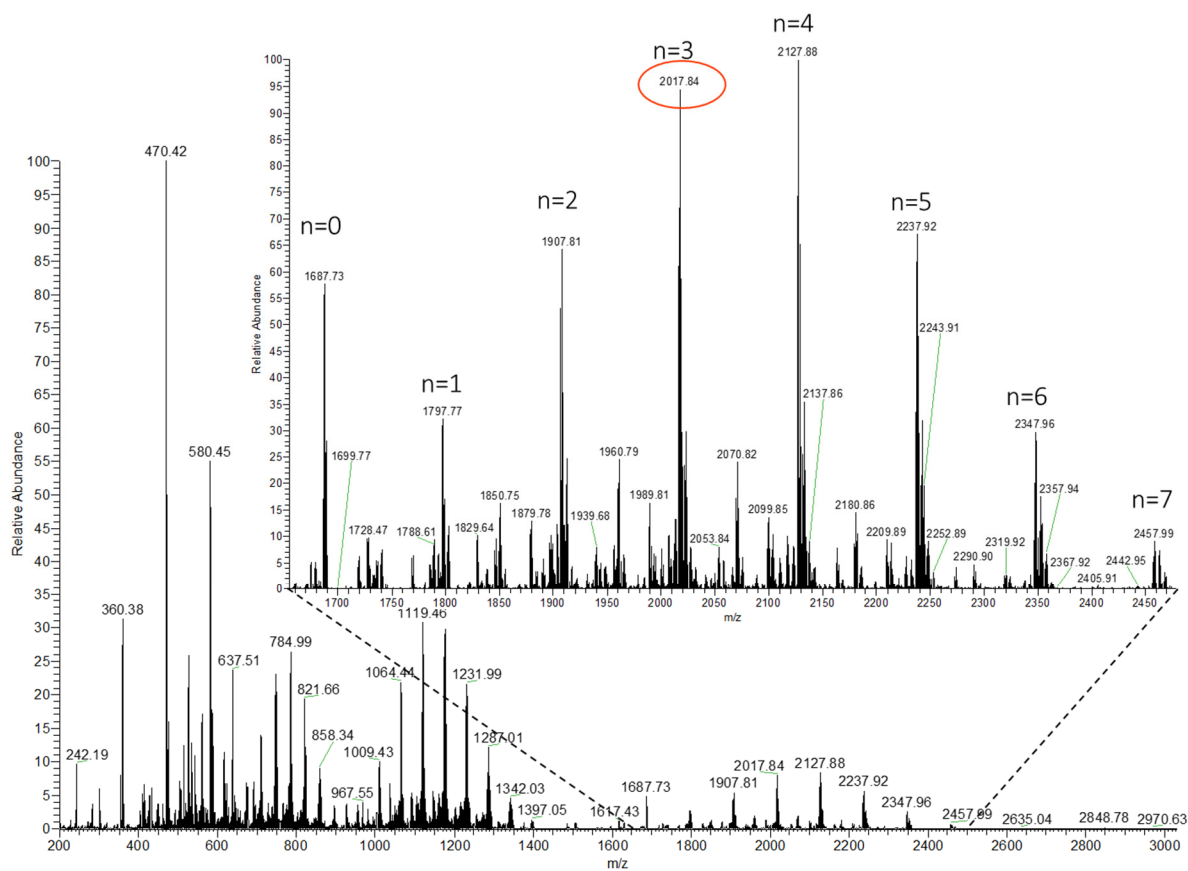
## Dendrimer **6a** ( $n = 2$ ) PPI-CB1



**Figure S20.** MS spectra of intermediate **4a** ( $n = 2$ ) Proposed molecular weight for **4a** ( $n = 2$ ) at full conversion is 1907.07. From the MS data, it can be concluded that the main species of functionalized dendrimer that can be observed in MS is indeed the  $n = 2$  species, but that also species with  $n$  ranging from 0 to 5 can be seen.



## Dendrimer **6a** ( $n = 3$ ) PPI-CB1

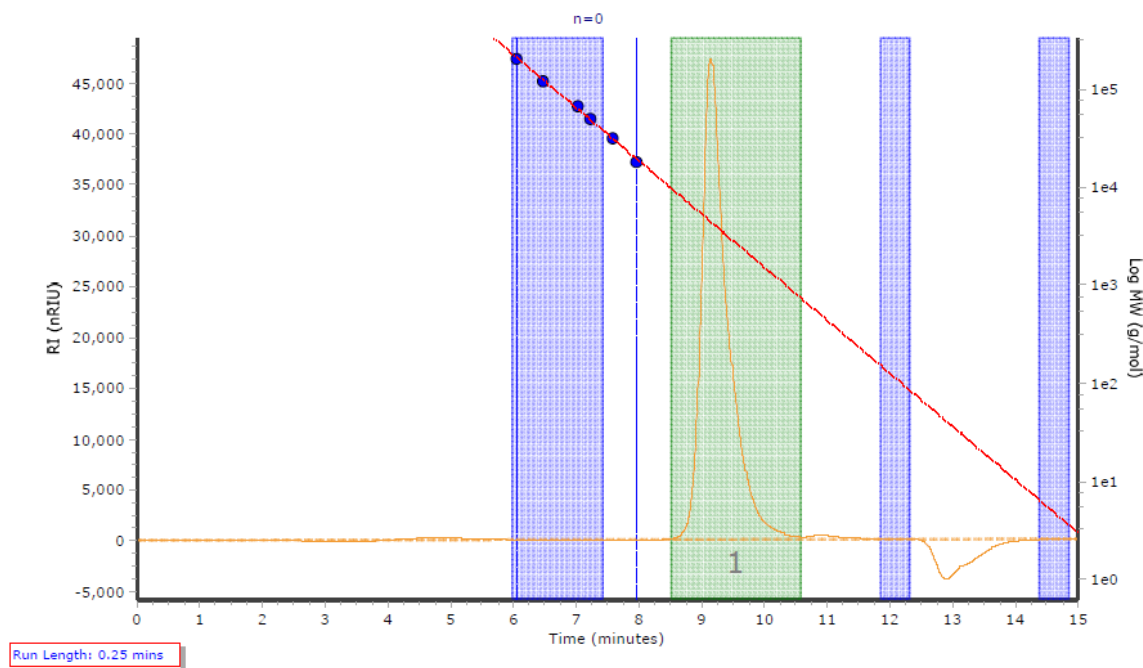


**Figure S21.** MS spectra of intermediate **4a** ( $n = 3$ ). Proposed molecular weight for **4a** ( $n = 3$ ) at full conversion is 2017.18. From the MS data, it can be concluded that the main species of functionalized dendrimer that can be observed in MS is indeed the  $n = 3$  and 4 species, but that also species with  $n$  ranging from 0 to 7 can be seen.

# Gel Permeation Chromatography data

## Dendrimer 3 PPI-CB1

### Chromatogram Plot



### Molecular Weight Averages

Peak	Mp (g/mol)	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	Mz+1 (g/mol)	Mv (g/mol)	PD
Peak 1	4567	3749	4133	4468	4770	4422	1.102

### Distribution Plot

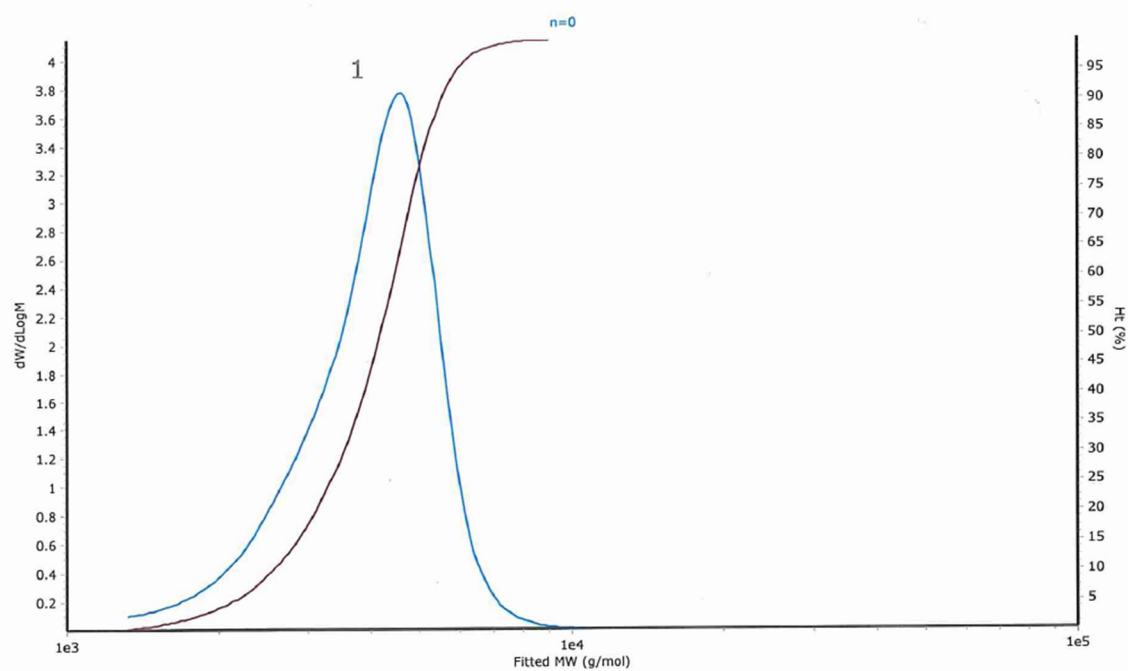
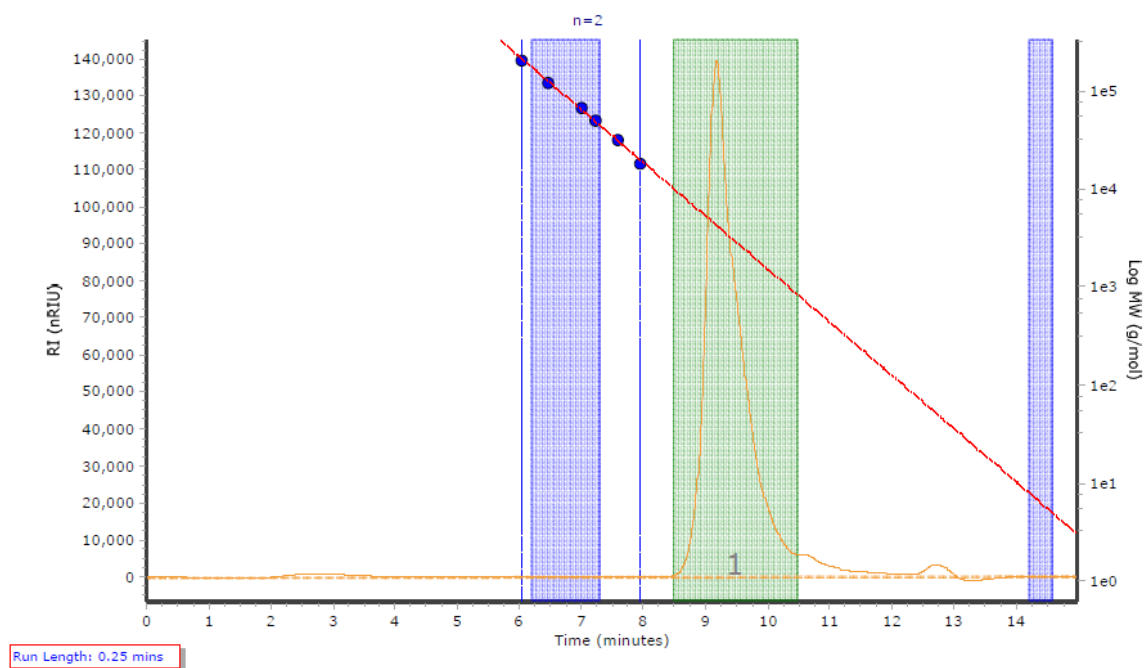


Figure S22. GPC trace and distribution plot of 3. Proposed molecular weight for 3 at full conversion is 3876.79.

## Dendrimer **6a** ( $n = 2$ ) PPI-CB1

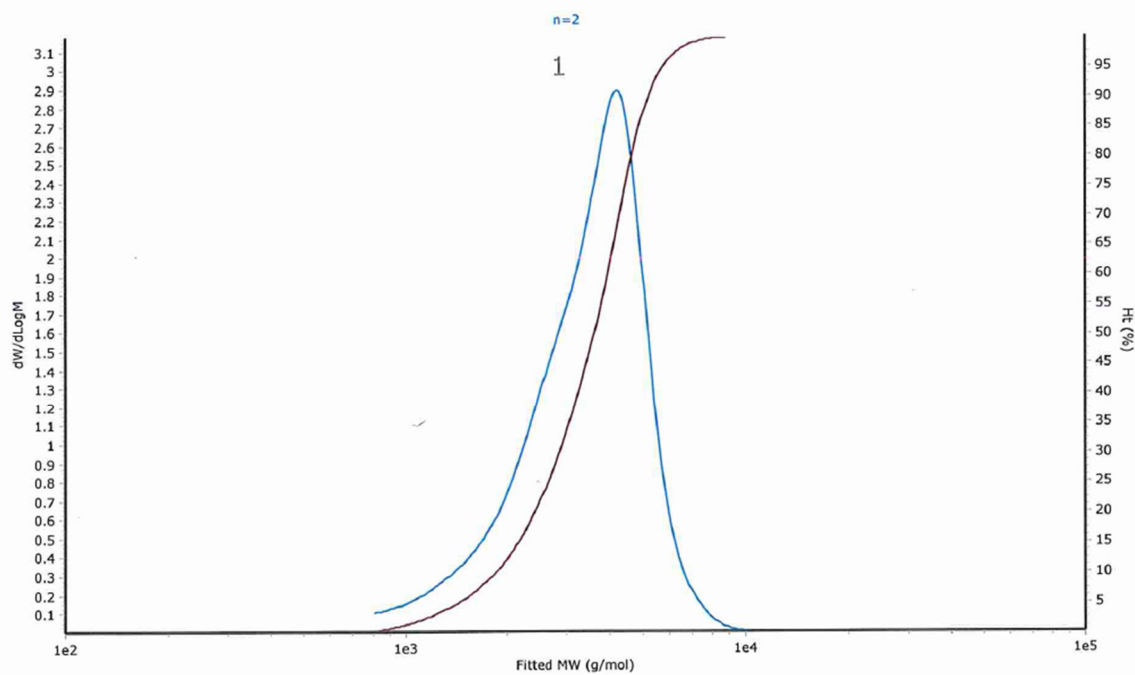
### Chromatogram Plot



### Molecular Weight Averages

Peak	Mp (g/mol)	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	Mz+1 (g/mol)	Mv (g/mol)	PD
Peak 1	4202	3011	3591	4077	4499	4012	1.193

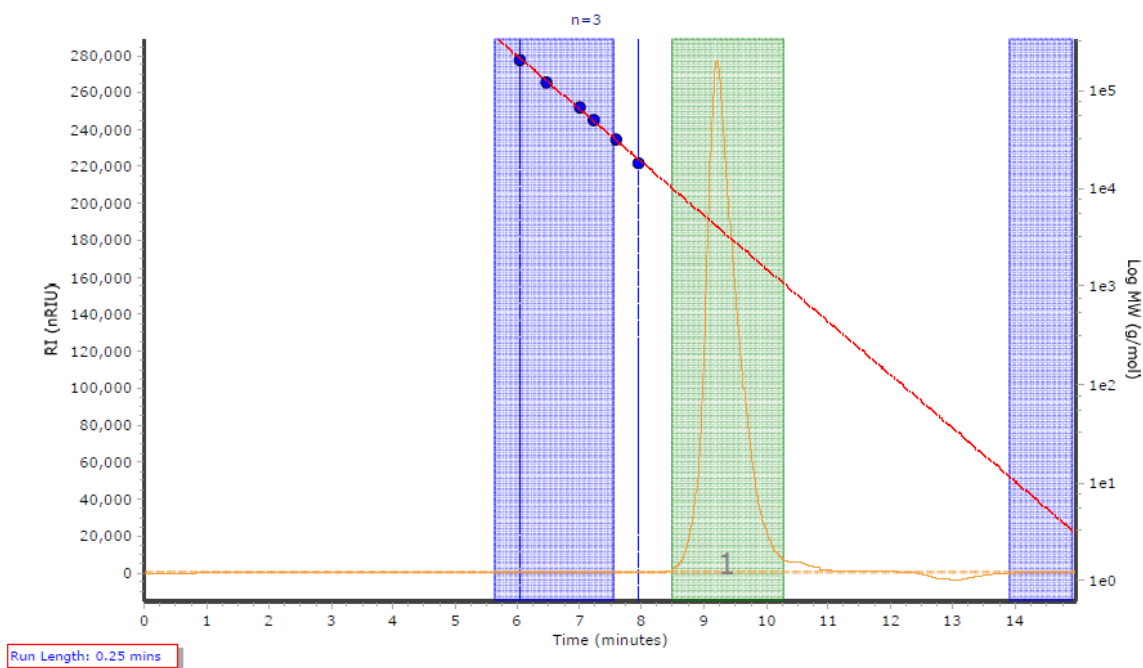
### Distribution Plot



**Figure S23.** GPC trace and distribution plot of intermediate **6a** ( $n = 2$ ). Proposed molecular weight for **6a** ( $n = 2$ ) at full conversion is 3924.83.

# Dendrimer **6a** ( $n = 3$ ) PPI-CB1

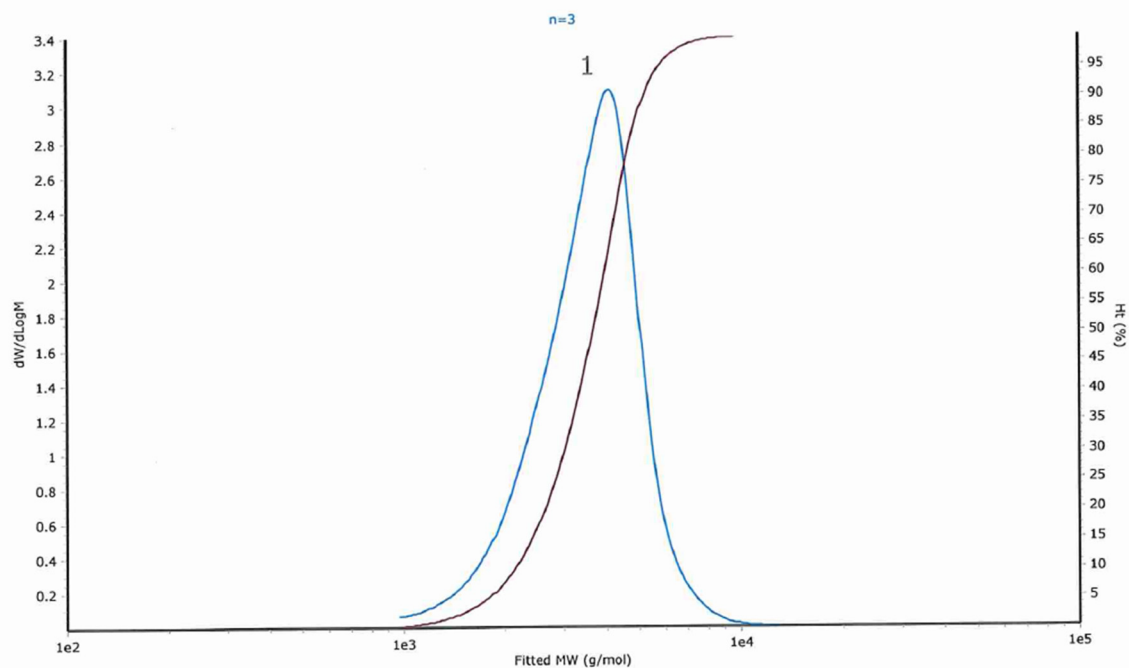
## Chromatogram Plot



### Molecular Weight Averages

Peak	Mp (g/mol)	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	Mz+1 (g/mol)	Mv (g/mol)	PD
Peak 1	4098	3244	3692	4114	4540	4053	1.138

### Distribution Plot



**Figure S24.** GPC trace and distribution plot of intermediate **6a** ( $n = 3$ ). Proposed molecular weight for **6a** ( $n = 3$ ) at full conversion is 3948.85.

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