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A new poly(propylene imine) dendron as potential convenient building-block in the construction of multifunctional systems

Hongmu Pan [†], Margaret E. Grow [†], Orla Wilson [‡], Marie-Christine Daniel [*]

Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, MD 21250, USA

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ABSTRACT

The synthesis of a novel third generation poly(propylene imine) dendron (PPI dendron) is described herein. The dendritic focal point was attached to a tetraethylene glycol spacer that was terminated with a Tmob-protected thiol in order to both avoid any side-reaction during functionalization of dendritic branches as well as enable convenient integration of the dendron into diverse complex systems. The protonation of the final PPI dendron at pH 4–5 is predicted to induce lysosomal degradation through the proton-sponge effect, and therefore enable cytosolic release. These features make the prepared dendron a very convenient building-block for use in the construction of larger systems, such as multifunctional drug delivery vehicles.

1. Introduction

During the past decades, dendrimers have achieved significant success in a variety of biomedical applications.[1–4] For instance, VivaGel® (Starpharma), a topical vaginal microbicide based on a polylysine dendrimer, is currently undergoing phase II clinical trials for the prevention of bacterial vaginosis recurrence. Additional examples are several dendrimer-based products[5] that received the CE mark, primarily for the traumatic or surgically-induced wound market: Ocuseal (a liquid ocular bandage), and Adherus™ Dural Sealant (used in cranial and spine surgeries to prevent cerebrospinal fluid leaks, and which has also successfully completed a U.S. pivotal clinical trial). Because of their nanoscale size and their branched structure, dendrimers allow for a longer circulation time in the body and they can carry a large payload.[1,4,6,7] They also present the advantage over conventional polymers to be well defined macromolecules with a polydispersity index equal to or close to 1, depending on the generation.[8] This last feature is particularly important in biomedical applications in order to ensure high batch-to-batch reproducibility of the therapeutic effects. Although the monodispersity and polyvalence of dendrimers represent great assets, one drawback is the fact that they usually display a single type of termini, and their multifunctionalization remains tedious and challenging.[9–13] Another limitation is the appearance of defects when reaching higher generations of dendrimers.

Dendrons differ from dendrimers in their structure by the presence of a focal point that is usually functionalized differently than the periphery, thus breaking the symmetry of the molecule.[8] This focal point allows for dendrons to be grafted onto other molecules (dendrons, dendritic cores, polymers…) or surfaces.[14–19] This is of particular interest for biomedical applications as dendrons can be incorporated easily and in a controlled manner in the preparation of multifunctional systems.[20–22] Indeed, optimal biomedical devices often require several different properties that are very challenging, or impossible, to find simultaneously on the same molecule; therefore, different entities need to be combined to fulfill the intended effect(s).[23–26] Although a variety of poly(amidoamine) dendrons (PAMAM dendrons)[17,22,27–31] and Fréchet-type dendrons[20,21,32–34] have been synthesized and utilized in the preparation of various complex systems, only few poly(propylene imine) (PPI) dendrons have been described.[35,36] Yet, the PPI dendritic scaffold presents several distinctive features, such as a more compact structure,[37] an enhanced solubility in a wider range of solvents due to its less polar interior[38] while still displaying water solubility, and predicted proton-sponge capability. The last feature has an important role in drug delivery as it is hypothesized to facilitate endosomal escape of the drug carrier after cell entry through receptor mediated endocytosis.

* Corresponding author. Tel.: +1 410 455 5601; fax: +1 410 455 2608; e-mail address: mdaniel@umbc.edu (M.-C. Daniel).

† Contributed equally to the work.

‡ Present address: Materials Science and Engineering, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21219, USA.

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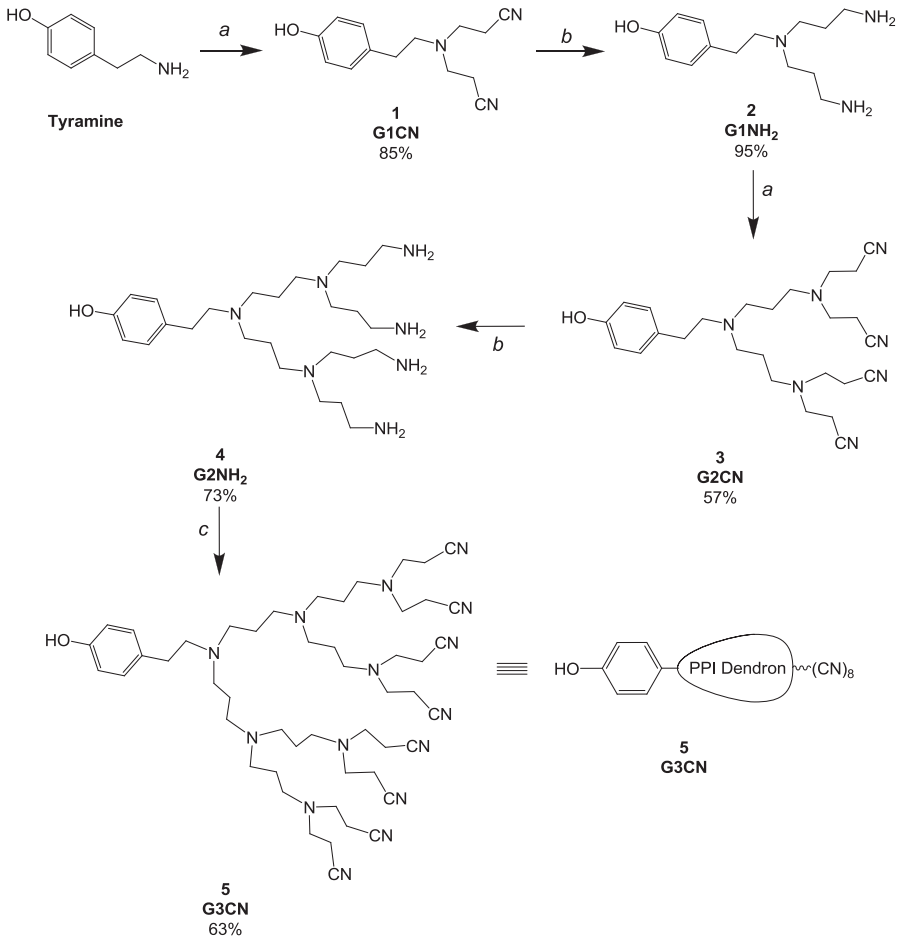
We report here the design, synthesis, and characterization of a novel dendron comprised of three parts: (1) a PPI dendron with a phenolic focal point; (2) a tetraethylene glycol (TEG) spacer at the focal point of the dendron; and (3) a protected thiol group at the extremity of the spacer. These components were chosen in order to impart the dendron with specific properties favoring its use in multifunctional systems for biomedical applications. First, the PPI and TEG parts are water-soluble entities, thus suitable for biological media. Second, the proton-sponge capability of PPI is a great asset in drug delivery, allowing for *endo*-lysosomal escape. Finally, the primary amine termini of the dendron can be used in a large variety of reactions (amide bond formation, nucleophilic substitution, Michael addition, imine bond formation, (thio)urea bond formation, etc.) with diverse medically relevant moieties.

2. Results/discussion

2.1. PPI dendron synthesis

Tyramine was chosen as the focal point for the preparation of the PPI dendron because it displays both a primary amine group, which can initiate the PPI dendritic growth, and a phenol group whose reactivity is different enough from the primary amine so that minimum competition occurs during the Michael addition of the acrylonitrile. The synthetic route is shown in Scheme 1. The conditions used for the Michael addition were similar to Voegtle's protocol[35] for the preparation of **G1CN** (**1**) and **G2CN** (**3**); however, for the preparation of **G3CN** (**5**), acrylonitrile could not be used as a solvent due to solubility issues, so methanol was used as the solvent and 40 equiv of acrylonitrile were added. The presence of the nitrile groups was evidenced by the distinctive FTIR band at 2248 cm^{-1}. Completion of the reaction was monitored by mass spectrometry. Addition of acrylonitrile to the phenol group was not observed, unless the reaction time was prolonged extensively or the acrylonitrile quantities were increased (data not shown). When addition to the phenol occurred, the ^1H NMR spectrum displayed an additional pair of aromatic doublets downfield from the peaks of the unreacted phenol. The ^1H NMR and mass spectrometry spectra of the third generation PPI dendron (**G3CN**, **5**) are shown in Fig. 1. The NMR signal at 2.8 ppm (in $CDCl_3$) is attributed to the protons of the eight methylene groups next to the nitrile termini.

The nitrile reduction to primary amines was carried out using borane dimethyl sulfide as the reducing agent.[39] This method readily reduced all nitrile groups into primary amines without noticeable side-reactions. To ensure complete reduction, three additions of 5 equiv of $BH_3 \cdot SMe_2$ per primary amine group were performed over the course of 8 h, which was followed by stirring at room temperature for about 24 h. After complete reduction, the excess borane was neutralized by very slow addition of cold methanol. Thus, $B(OMe)_3$ was formed as a side product and proved to be difficult to remove from the dendron, with the corresponding signal appearing in the ^1H NMR spectrum at about 3.4 ppm. It is possible that some



Reagents and Conditions: a, Acrylonitrile, AcOH (cat.), reflux, 24 h; *b,* $BH_3 \cdot Me_2S$, anhyd. THF, Ar, RT, 24 h; *c,* Acrylonitrile, MeOH, reflux, 48 h.

Scheme 1. PPI dendron synthesis.

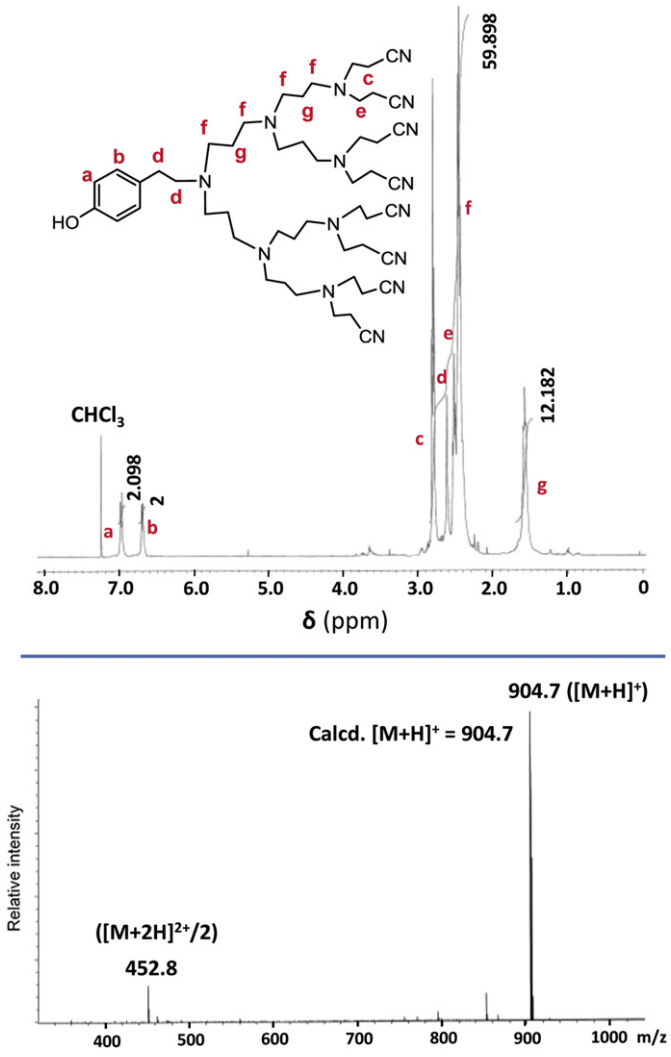


Fig. 1. Top: ^{1}H NMR of **G3CN** PPI dendron in $CHCl_3$; bottom: ESI mass spectrum of **G3CN** PPI dendron.

$B(OMe)_3$ gets 'entrapped' inside the dendron through coordination by the amines, which renders its removal difficult. We could solve this problem by refluxing the reduced dendron overnight in fresh methanol and isolating the dendron into water. Other trials for the nitrile reduction were performed using various methods and reducing agents, including $CoCl_2/NaBH_4$,[35] Raney-Ni,[40,41] and Raney-Co,[42] but they either resulted in incomplete reduction of all eight nitrile groups or in partial degradation of the product. Additionally, although it needs to be carried out under inert atmosphere, the reduction using $BH_3{\cdot}SMe_2$ has the advantage over other techniques of not requiring any specialized equipment and of using mild conditions (room temperature, atmospheric pressure). **G1NH$_2$** (**2**) and **G2NH$_2$** (**4**) were obtained with high yields (95% and 73%, respectively), after purification by extraction into water, washes with hexane and diethyl ether, and lyophilization. Dendrons **2** and **4** were characterized by FTIR, NMR, and mass spectrometry. The stretching band of the nitrile groups disappears in the FTIR spectra and a broad band appears at frequencies around 2800–3500 cm^{-1}, indicative of the primary amino groups. In the ^{1}H NMR spectra, the signal that corresponded to the methylene groups next to the nitrile functions (at 2.8 ppm) is shifted upfield to about 1.5 ppm after complete conversion of the nitriles to primary amines, and the $-CH_2-$ formed next to $-NH_2$ give a signal at 2.7–2.9 ppm depending on the deuterated solvent (D_2O, CD_3OD or $CDCl_3$).

G3CN was reduced to **G3NH$_2$** only after attachment to the spacer in order to avoid competition between primary amines and the phenol group as the role of nucleophiles, which would lead to undesired coupling of the spacer to the dendritic branches.

2.2. Spacer synthesis

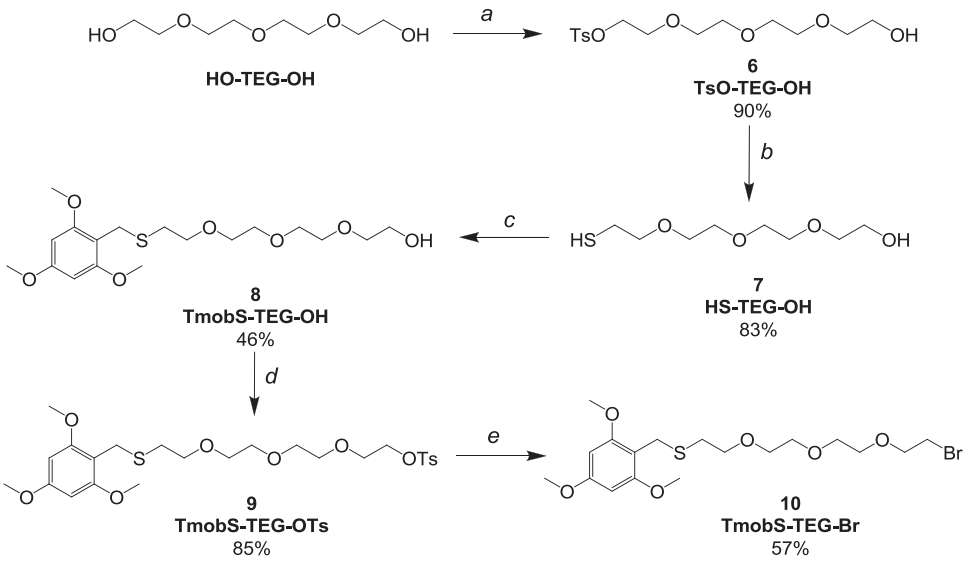
In order to facilitate anchoring of the dendron to a variety of other molecules or surfaces, we attached a spacer to the phenolic focal point of the **G3CN** dendron. Tetraethylene glycol (TEG) was selected as the spacer for a number of reasons: (1) its chemistry is well established, (2) it is water-soluble, thus facilitating its use in biological media; (3) it is a monodisperse molecule, unlike high molecular weight poly(ethylene glycols), thus preserving the monodispersity of the spacer-dendron construct to be prepared; (4) other oligo(ethylene glycols) can easily be used in place of TEG by following the same synthetic protocols as used for TEG.

TEG was functionalized with a bromine group at one end and a thiol group at the other end to prepare for attachment to the dendron and to facilitate grafting to a variety of other systems, respectively. Scheme 2 displays the steps involved in the modification of TEG. Monothiolated TEG (**HS-TEG-OH**, **7**) was first prepared as previously described in the literature,[43] using monotosylated TEG (**TsO-TEG-OH**, **6**) as an intermediate followed by addition of thiourea and basic hydrolysis. Since **HS-TEG-OH** is readily prone to oxidation in air over time, (and also to avoid thiol reactivity during subsequent reactions) the thiol group was protected. 2,4,6-Trimethoxybenzyl (Tmob) was chosen as the protective group for its stability under mild acidic conditions, strong basic conditions, and reductive conditions. Moreover, its deprotection can be achieved in high yields using specific conditions (5% TFA and cation scavenger, vide supra). **TmobS-TEG-OH** (**8**) was prepared in the presence of 10% TFA in dichloromethane and obtained in 46% yield after flash column chromatography. The presence of the protection is evidenced by the ^{1}H NMR singlet at 6 ppm, corresponding to the aromatic protons of Tmob group. Next, **TmobS-TEG-Br** (**10**) was synthesized (using **TmobS-TEG-OTs** (**9**) as an intermediate) to provide an excellent leaving group for attachment to **G3CN** (**5**) dendron.

2.3. Coupling of spacer and dendron

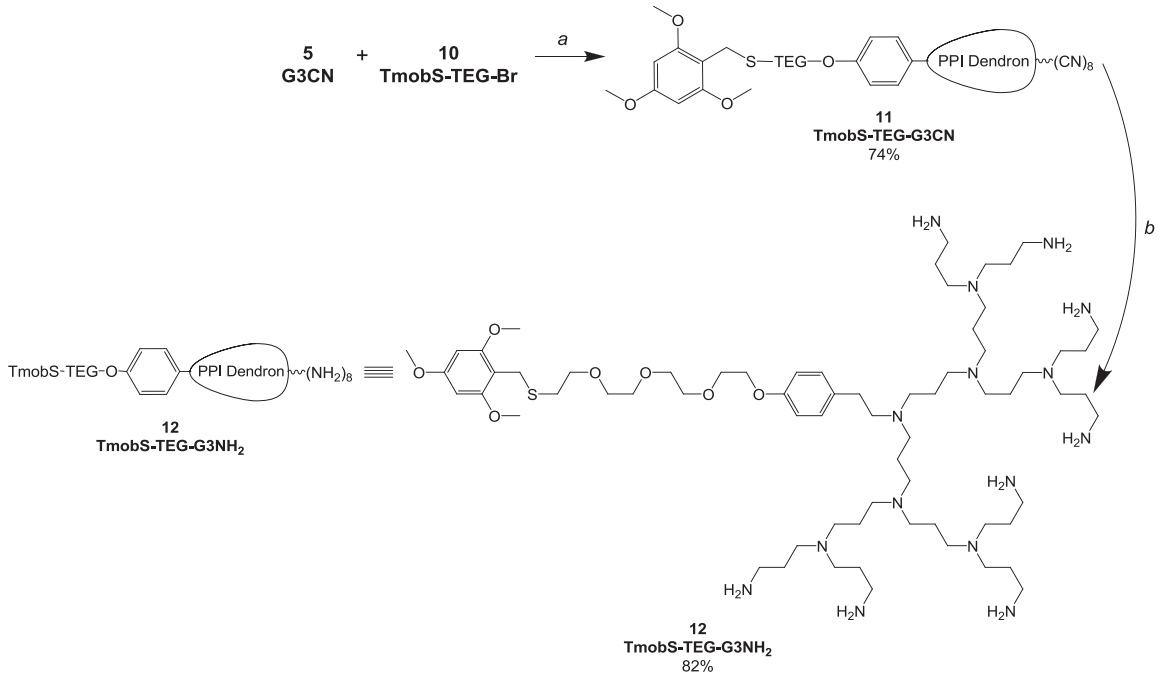
The TEG spacer was attached to the PPI dendron through nucleophilic substitution at the phenolic focal point of the dendron (Scheme 3). Several trials using K_2CO_3 and DMF resulted in very long reaction times (about 7 days). On the other hand, complete coupling was achieved in about 48 h at room temperature using NaH as the base and THF as the solvent. Furthermore, no deprotection of the thiol occurred, as verified in the ^{1}H NMR spectrum (Fig. S9, Supplementary data) by the singlet at 6 ppm still indicating an integration of two protons for the two aromatic protons of Tmob group (relative to the doublets of the phenolic protons, around 7 ppm, also indicating two protons). **TmobS-TEG-G3CN** (**11**) was obtained as viscous light-yellow oil in 74% yield. As a note, the coupling reaction was also performed with **TmobS-TEG-OTs** as the starting material, but required longer reaction times.

After coupling to the TEG spacer, the nitrile termini of the dendron were reduced to primary amines to form dendron **12**, following a similar procedure as when reducing **G1CN** and **G2CN** dendrons, i.e., using borane dimethyl sulfide (Scheme 3). Again, as shown in the ^{1}H NMR spectrum of Fig. 2, the Tmob protection remained intact under these strong reductive conditions. Interestingly, we noticed that **TmobS-TEG-G3NH$_2$** (**12**) was not only soluble in water and methanol, but also readily dissolved in chloroform, unlike **G2NH$_2$**; thus, the presence of the spacer provides the additional advantage of decreasing the overall polarity of the dendron, which enables its use in



Reagents and Conditions: a, TsCl, NaOH, THF, 0°C, 2 h; *b,* i) Thiourea, Abs. EtOH, reflux, Ar, 24 h, ii) NaOH in EtOH/H_2O, Abs. EtOH, reflux, Ar, 2.5 h; *c,* 2,4,6-trimethoxybenzalcohol, 10% TFA in CH_2Cl_2, Ar, RT, 5 h; *d,* TsCl, NaOH, THF, RT, 24 h; e, LiBr, acetone, reflux, 24 h.

Scheme 2. Spacer synthesis.



Reagents and Conditions: a, NaH, anhyd. THF, Ar, RT, 48 h; *b,* $BH_3{\cdot}Me_2S$, anhyd. THF, Ar, RT, 24 h.

Scheme 3. Synthesis of the TEGylated PPI dendron.

a larger variety of solvents. Mass spectrometry of **12** was carried out using atmospheric pressure chemical ionization (APCI) because of difficulties encountered in obtaining a signal using electrospray ionization (ESI). Because APCI is a slightly harsher technique than ESI, a few fragmentations occurred and are thus observed in the spectrum. The lost fragments correspond to $(CH_2)_2NH$, $(CH_2)_2NH+(CH_2)_3NH$, $(CH_2)_2N[(CH_2)_2NH]_2$ and $3\times(CH_2)_3NH$.

2.4. Thiolated PPI dendron

As a control reaction, deprotection of Tmob was attempted on dendron **11** (Scheme 4). Using 5% TFA in the presence of a cation scavenger (Et_3SiH in this case, but L-cysteine is another possible scavenger[44]), complete deprotection was afforded at room temperature overnight, yielding over 90% of **HS-TEG-G3CN** (**13**) after

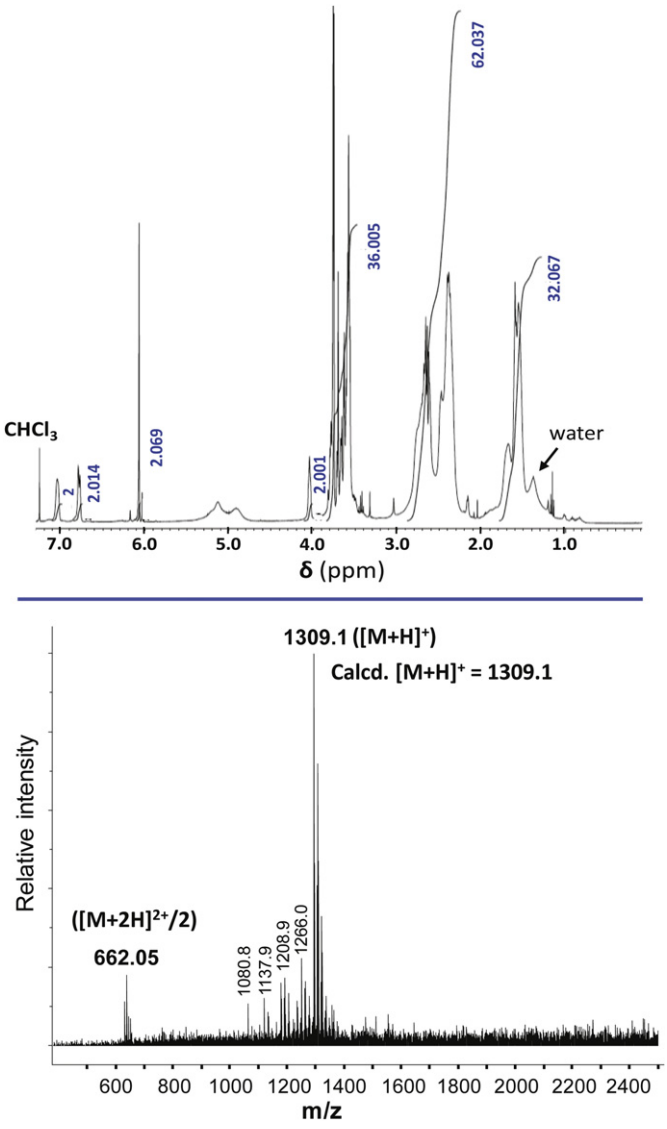


Fig. 2. Top: ^{1}H NMR of **TmobS-TEG-G3NH$_2$** (**12**) in $CHCl_3$; bottom: APCI mass spectrum of **TmobS-TEG-G3NH$_2$** (**12**).

purification. While protected dendron **11** is soluble in chloroform, once the thiol group is deprotected, its solubility in $CHCl_3$ is negligible and it becomes solely soluble in polar solvents, such as water, methanol or acetone. ^{1}H NMR in deuterated acetone (Fig. S11, Supplementary data) allows for the observation of the thiol proton, with a signal at 1.26 ppm. Also, the signal at 6 ppm, corresponding to the Tmob aromatic protons, disappeared, as well as the singlet from the Tmob methoxy groups (at 3.74 ppm) and the singlet from Tmob benzylic protons (at 3.68 ppm). The deprotection was further confirmed by ESI mass spectrometry (Fig. S12, Supplementary data).

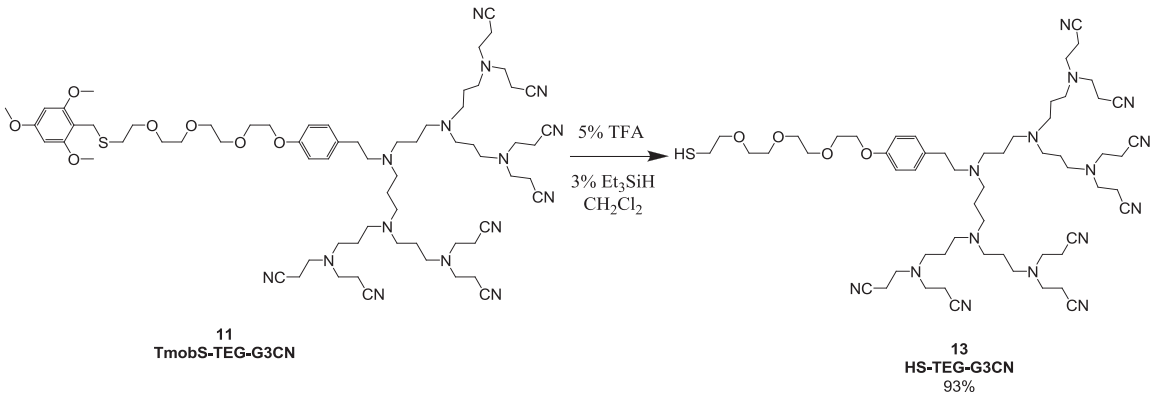
The stability of the thiol group in dendron **13** was investigated: indeed, **HS-TEG-OH** is known to oxidize after a few days in air;[45] additionally, the presence of trace transition metal (in buffers, reagents etc.) can catalyze thiol oxidation.[46,47] Thus, after isolation and characterization, **HS-TEG-G3CN** (**13**) was monitored for its stability under ambient atmosphere for a period of 15 days. No disulfide formation was noticed by mass spectrometry over this time period. Furthermore, a control experiment was carried out by adding iron (III) chloride to an aqueous solution of dendron **13** and by assessing the oxidation of the thiol group (Supplementary data): an Ellman's test revealed that 94% of dendron **13** remained in the thiol form after 20 min incubation with $FeCl_3$ (the same conditions led to complete thiol oxidation in Ref. 46). This observed increase in thiol stability after coupling to the dendron suggests that the dendron sterically 'protects' the thiol from disulfide formation.

2.5. Potential for proton-sponge effect

Similar to polyethyleneimine (PEI) structures,[48] the PPI dendrons presented herein display a number of tertiary amines. PEI has demonstrated the ability to induce endosomal escape by the 'proton-sponge effect'[49] due to the protonation of its tertiary amines at low pH. Thus, we verified the protonation behavior of the tertiary amines of our PPI dendron by studying the ζ potential of **11** at a pH range from 2 to 7. By studying **11** instead of **12**, we avoided interferences from the protonation of the terminal primary amines of **12**. As shown in Fig. 3, a decrease in pH from 7 to 5 induces only a slight increase in the ζ potential of **11**; however, the overall charge of **11** dramatically increases when the pH decreases from 5 to 4, suggesting a high degree of protonation when dropping the pH below 5. This pH of 4–5 corresponds to lysosomal pH, and consequently, protonation of our PPI dendron at this pH is likely to induce osmotic swelling and lysis of the lysosome through the proton-sponge effect (as already observed using PEI[50]), and hence enable release of the dendron into the cytosol.[51] This would constitute a significant benefit in the field of drug delivery. Further experiments will be carried out in order to check this hypothesis.

3. Conclusion

We prepared a PPI dendron of generation 3 (displaying eight branches) with its focal point coupled to a Tmob-protected thiolated TEG. This dendron presents several attractive features: (1) the protected form (dendron **12**) is soluble in a variety of solvents



Scheme 4. Deprotection of **TmobS-TEG-G3CN** (**11**).

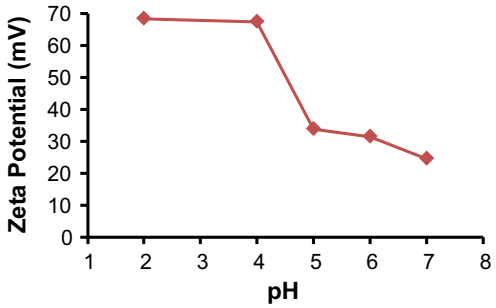


Fig. 3. Protonation of **TmobS-TEG-G3CN** (**11**) at different pHs: ζ potential of **TmobS-TEG-G3CN** (**11**) versus pH in water.

ranging from chloroform to water, which will ease further conjugation of the dendritic branches with diverse moieties; (2) the deprotected form (dendron **13**) is stable against thiol oxidation in air, so no specific precautions are needed during its handling or short-term storage; (3) the thiolated spacer will assist with grafting of the PPI dendron onto other constructs, such as other dendrons, surfaces, nanoparticles etc.; (4) the tertiary amines of the PPI dendron interior show potential to act as proton-sponges for the biological purpose of endosomal escape. Overall, this novel PPI dendron represents a suitable candidate for biomedical use, and displays features that facilitate its use as a building-block in the construction of diverse multifunctional systems.

4. Experimental section

4.1. Materials

All solvents and reagents were purchased from commercial sources and used without purification except where specified. Anhydrous solvents (THF, CH_2Cl_2, CH_3CN, MeOH, and DMF) were retrieved from the solvent purification system (mBraun MB-SPS) and stored under a nitrogen atmosphere shortly before use.

Proton and carbon nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR, respectively) were obtained using a JEOL ECX 400 MHz NMR spectrometer, operated at 400 and 100 MHz, respectively. Chemical shifts (δ) are in parts per million using an internal standard to TMS as the reference, set to 0.00 ppm. Abbreviations used in proton data are as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), dd (doublet of doublets), dt (doublet of triplet), m (multiplet), br (broad). NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). Mass spectra were recorded at the UMBC Molecular Characterization and Analysis Complex (MCAC) (Catonsville, MD). Fourier transform infrared spectroscopy (FTIR) was performed using a Perkin Elmer Paragon 500 with a horizontal ATR accessory and ZnSe crystal flat plate.

Thin layer chromatography on silica gel (SiO_2) was performed using Whatman Partisil K5 150 Å glass plates (250 μm) without preadsorbent zone. Column chromatography was performed using silica gel (63–200 μm) from Uline, Inc., and eluted with the indicated solvent system.

Reactions at 'room temperature' (RT) were conducted at ambient laboratory temperature (20–26 °C). Distillation of solvents on a rotary evaporator at 20–50 °C and 25–15 mmHg is referred to as 'removal of solvent under reduced pressure'; 'in vacuo' refers to pressures of 1.0–0.25 mmHg.

4.2. Syntheses of dendrons

4.2.1. ***G1CN*** *(**1**).* Acrylonitrile (100 mL), tyramine (1.373 g, 10.01 mmol) and glacial acetic acid (1.25 mL, 20 mmol per primary amino function) was heated to reflux for 24 h. After 24 h, the solution was cooled to room temperature, and excess acrylonitrile was removed under reduced pressure. The resulting oil was dissolved in chloroform, washed with ammonium hydroxide and water, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting white solid was recrystallized using chloroform to yield the product as a white solid (2.077 g, 85% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.04 (d, J=8.2 Hz, 2H), 6.75 (d, J=8.7 Hz, 2H), 2.88 (t, J=6.9 Hz, 4H), 2.65–2.77 (m, 4H), 2.39 (t, J=6.9 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 154.4, 131.6, 130.1, 118.8, 115.6, 56.1, 50.1, 33.6, 17.4. IR ν_{max} (neat) 2245 cm^{-1} (nitrile). HRMS (ESI) calcd for $C_{14}H_{18}N_3O$ ($M+H^+$) 244.1444, observed 244.1448.

4.2.2. ***G1NH$_2$*** *(**2**).* To a solution of **1** (1.02 g, 4.19 mmol) in anhydrous THF (20 mL) under argon was added borane dimethyl sulfide complex (4.5 mL, 47 mmol, 5 equiv per nitrile group) using a syringe. The reaction mixture was stirred at room temperature until gelification was evident. The borane dimethyl sulfide complex was added in two more additions over the course of one day. The mixture was allowed to stir overnight. Methanol was added slowly at 0 °C until no further bubbling or reaction was observed. The solvent was removed under reduced pressure, after which fresh methanol (50 mL) was added to the residual oil and heated to reflux overnight. Upon cooling to room temperature, the solvent was removed under reduced pressure. The oily residue was taken up in water (10 mL) and washed with diethyl ether (3×5 mL), hexane (3×5 mL), and ethyl acetate (1×5 mL), and lyophilized to yield 1.005 g of the product as a clear colorless oil (95% yield). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 6.91 (d, J=8.2 Hz, 2H), 6.59 (d, J=8.7 Hz, 2H), 2.43–2.55 (m, 12H), 1.53 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 157.2, 132.3, 130.8, 116.5, 62.9, 52.9, 41.2, 33.2, 22.4. HRMS (ESI) calcd for $C_{14}H_{26}N_3O$ ($M+H^+$) 252.2070, observed 252.2075.

4.2.3. ***G2CN*** *(**3**).* Acrylonitrile (50 mL), **2** (3.076 g, 12.24 mmol) and glacial acetic acid (2.5 mL, 20 mmol per primary amino function) were added together at room temperature and heated to reflux for 48 h. After 48 h, the solution was cooled to room temperature. Excess acrylonitrile was removed under reduced pressure. The resulting oil was dissolved in chloroform, washed with ammonium hydroxide and water, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude yellow oil was purified via column chromatography (80:10:1 $CHCl_3$/MeOH/NH_4OH) to yield 3.21 g of the product as a cloudy viscous oil (57% yield): R_f=0.472 (SiO_2, 80:10:1 $CHCl_3$/MeOH/NH_4OH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.00 (d, J=8.2 Hz, 2H), 6.72 (d, J=8.2 Hz, 2H), 2.76 (t, J=6.9 Hz, 8H), 2.63 (br s, 4H), 2.52 (t, J=7.3 Hz, 4H), 2.46 (t, J=6.9 Hz, 4H), 2.41 (t, J=6.9 Hz, 8H), 1.56 (quin, J_1=7.3 Hz, J_2=6.9 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 154.7, 132.2, 130.0, 119.1, 115.6, 55.7, 51.6, 51.5, 49.7, 32.3, 24.9, 17.0. IR ν_{max} (neat) 2249 cm^{-1} (nitrile). HRMS (ESI) calcd for $C_{26}H_{38}N_7O$ ($M+H^+$) 464.3132, observed 464.3133.

4.2.4. ***G2NH$_2$*** *(**4**).* To a solution of **3** (0.270 g, 0.582 mmol) in anhydrous THF (20 mL) under argon was added borane dimethyl sulfide complex (1.1 mL, 11.65 mmol, 5 equiv per nitrile group) using a syringe. The reaction mixture was stirred at room temperature until gelification was evident. The borane dimethyl sulfide complex was added in two more additions over the course of the day. The mixture was allowed to stir for one additional day at room temperature. Methanol was added slowly at 0 °C until no further bubbling or reaction was observed. The solvent was removed under reduced pressure, after which fresh methanol (50 mL) was added to the residual oil and heated to reflux overnight. Upon cooling to room temperature, the solvent was removed under reduced pressure. The oily residue was taken up in 15 mL water and washed with hexane (2×10 mL) and diethyl ether (2×10 mL) and lyophilized overnight to yield the product as a white fluffy powder (0.203 g,

73% yield). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.00 (d, 2H), 6.69 (d, 2H), 2.81–2.43 (m, 28H), 1.69–1.56 (br m, 12H). ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 155.5, 131.1, 129.4, 115.0, 61.5, 55.7, 51.8, 51.3, 39.2, 31.9, 27.3, 23.5. HRMS (ESI) calcd for $C_{26}H_{54}N_7O$ ($M+H^+$) 480.4384, observed 480.4391.

4.2.5. ***G3CN*** *(**5**).* To a solution of **4** (6.38 g, 13.31 mmol) in methanol (100 mL) was added acrylonitrile (50 mL, 56 equiv). The solution was heated to reflux for 48 h. The reaction was then cooled to room temperature and the solvent was removed under reduced pressure. The residue was taken up in deionized water and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude oil was washed with hexane and diethyl ether until no further impurities were observed via ^{1}H NMR to yield a clear yellow oil (7.54 g, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.01 (d, 2H), 6.73 (d, 2H), 2.20–2.92 (m, 60H), 1.51–1.64 (br m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 155.1, 131.7, 129.9, 119.1, 115.7, 55.9, 52.2, 51.7, 51.5, 49.6, 32.4, 24.9, 24.3, 17.0. IR ν_{max} (neat) 2247 cm^{-1} (nitrile). HRMS (ESI) calcd for $C_{50}H_{78}N_{15}O$ ($M+H^+$) 904.6508, observed 904.6526.

4.3. Synthesis of spacer

4.3.1. ***TsO-TEG-OH*** *(**6**).* To a solution of tetraethylene glycol (100.1 g, 515.2 mmol) in 230 mL THF was added a solution of sodium hydroxide (6.89 g, 172.3 mmol) dissolved in 20 mL deionized water. The mixture was cooled to 0 °C and toluene sulfonyl chloride (9.81 g, 51.5 mmol) in 20 mL THF was added dropwise. The reaction was allowed to stir at 0 °C for 2 h. The solution was poured into deionized water and the aqueous layers were separated and extracted with dichloromethane. The organic layers were combined and washed with water, dried over sodium sulfate, filtered and concentrated under reduced pressure to yield 16.17 g of product as a clear colorless oil (90% yield, based on the toluene sulfonyl chloride): R_f=0.634 (SiO_2, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.79 (d, 2H), 7.33 (d, 2H), 4.16 (t, 2H), 3.55–3.72 (m, 14H), 2.44 (s, 3H). Spectral data agreed with literature values.[52]

4.3.2. ***HS-TEG-OH*** *(**7**).* Absolute ethanol (65 mL) and thiourea (0.471 g, 6.19 mmol) were added to dried **6** (2.03 g, 5.83 mmol) and allowed to reflux under argon for 24 h. After 24 h, the reaction was cooled to room temperature and sodium hydroxide (0.700 g, 17.51 mmol) in absolute ethanol/water (9:1 v/v, 8.5 mL) was added. The reaction was heated to reflux under argon for 3 h. The reaction was then cooled to room temperature, acidified with concentrated hydrochloric acid to pH 2, filtered to remove the salts, and concentrated under reduced pressure. The crude oil was purified via column chromatography (10:1 EtOAc/absolute ethanol) to obtain 1.022 g of the product as a pale yellow oil (83.3% yield, 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.58–3.75 (m, 14H), 2.70 (dt, 2H), 2.46 (broad s, 1H), 1.62 (t, 1H). Spectral data agreed with literature values.[45,53]

4.3.3. ***TmobS-TEG-OH*** *(**8**).* To a solution of **7** (870 mg, 4.14 mmol) in 40 mL of anhydrous dichloromethane under nitrogen was slowly added trifluoroacetic acid (4 mL) at room temperature. The solution was stirred for 3 min until 2,4,6-trimethoxybenzyl alcohol (984 mg, 4.97 mmol) in 5 mL of anhydrous dichloromethane was added. [2,4,6-Trimethoxybenzyl alcohol was previously prepared by following the procedure from the literature.[54]] The reaction mixture was stirred for 2 h at room temperature after which saturated $NaHCO_3$ was slowly added to neutralize the solution. The resulting mixture was separated and the water layer was washed with dichloromethane (3×50 mL). The combined organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The product (743 mg, 1.90 mmol, 46% yield) was yielded as a yellow oil through flash column chromatography (2:1 EtOAc/Hex): R_f=0.208 (SiO_2, 2:1 EtOAc/Hex); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.98 (s, 2H), 3.67 (s, 6H), 3.65 (s, 3H), 3.61 (s, 2H), 3.57 (br, 2H), 3.51–3.43 (m, 12H), 2.55 (t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.0, 158.5, 107.6, 90.3, 77.2, 72.3, 70.6, 70.3, 70.2, 70.1, 69.9, 61.3, 55.5, 55.1, 30.7, 23.5. HRMS (ESI) calcd for $C_{18}H_{30}O_7S$ ($M+H^+$) 391.1785, observed 391.1762.

4.3.4. ***TmobS-TEG-OTs*** *(**9**).* To a solution of **21** (538 mg, 1.38 mmol) in THF (50 mL) was added 2 mL 2 M NaOH at room temperature. The solution was stirred for 30 min until TsCl (688 mg, 4 mmol) was added. The reaction mixture was stirred overnight before addition of 30 mL of water. The resulting mixture was separated and the water layer was washed with ethyl acetate (3×30 mL). The combined organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The product (638 mg, 1.17 mmol, 85% yield) was yielded as an oil through flash column chromatography (2:1 EtOAc/Hex): R_f=0.65 (SiO_2, 2:1 EtOAc/Hex); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.62 (d, 2H), 7.17 (d, 2H), 5.97 (s, 2H), 3.98 (t, 2H), 3.65–3.40 (m, 23H), 2.52 (t, 2H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.4, 158.7, 144.8, 132.9, 129.9, 127.9, 107.9, 90.5, 70.8, 70.6, 70.5, 70.5, 70.1, 69.4, 68.6, 30.9, 23.8, 21.5. HRMS (ESI) calcd for $C_{25}H_{37}O_9S_2$ ($M+Na^+$) 567.1693, observed 567.1686.

4.3.5. ***TmobS-TEG-Br*** *(**10**).* To a solution of **9** (420 mg, 0.77 mmol) in 40 mL acetone was added LiBr (0.90 g, 10.4 mmol) at room temperature and then heated to reflux overnight. The solution was concentrated under reduced pressure and the resulting oil was redissolved in ethyl acetate (80 mL) and washed with water and brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The product (198 mg, 0.44 mmol, 57% yield) was yielded as a pale yellow oil through flash column chromatography (2:1 Hex/EtOAc): R_f=0.3 (SiO_2, 2:1 Hex/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.05 (s, 2H), 3.74–3.72 (m, 11H), 3.68 (s, 2H), 3.58–3.54 (m, 10H), 3.39 (t, 2H), 2.62 (t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.3, 158.8, 108.1, 90.6, 71.2, 71.0, 70.7, 70.6, 70.2, 55.8, 55.4, 31.0, 30.6, 23.9. HRMS (ESI) calcd for $C_{18}H_{30}BrO_6S$ ($M+H^+$) 453.0941, observed 453.0940.

4.4. Dendron-spacer

4.4.1. ***TmobS-TEG-G3CN*** *(**11**).* To a solution of **5** (230 mg, 0.25 mmol) in 10 mL of anhydrous THF under argon was added sodium hydride (60% dispersion in mineral oil) (23 mg, 0.58 mmol). The mixture was stirred at room temperature for several hours until the solution turned yellow. At this point, the yellow mixture was transferred (using cannula) to a solution of **10** (410 mg, 0.90 mmol) in anhydrous THF and the resulting solution was stirred at room temperature for 48 h. 1 mL of methanol was then added to neutralize the excess of sodium hydride. The solvent was evaporated under reduced pressure after being stirred for 15 more minutes. The oily residue was washed with diethyl ether (3×5 mL). The washed oil was dissolved in a minimum of ethyl acetate and reprecipitated with cold diethyl ether. The residue was dried in vacuo to yield the product as light-yellow viscous oil (236 mg, 0.18 mmol, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.06 (d, 2H), 6.80 (d, 2H), 6.03 (s, 2H), 4.00 (t, 2H), 3.72–3.71 (m, 11H), 3.66 (s, 2H), 3.62–3.51 (m, 10H), 2.75–2.71 (m, 14H), 2.61–2.57 (m, 8H), 2.47–2.32 (m, 40H), 1.52–1.48 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 160.3, 158.8, 157.1, 132.9, 129.6, 119.0, 114.6, 108.0, 90.6, 70.6, 70.5, 70.4, 70.3, 69.9, 69.5, 67.2, 55.8, 55.6, 55.2, 51.9, 51.9, 51.4, 51.2, 49.3, 32.2, 30.8, 24.7, 24.3, 23.6, 16.6. HRMS (ESI) calcd for $C_{68}H_{106}N_{15}O_7S$ ($M+H^+$) 1276.8115, observed 1276.8170.

4.4.2. ***TmobS-TEG-G3NH$_2$*** *(**12**).* To a solution of **11** (94 mg, 0.074 mmol) in 5 mL of anhydrous THF under argon was slowly

added borane dimethyl sulfide complex (1.2 mL, 11.84 mmol) at room temperature. The mixture was stirred at room temperature for 2 h until another addition of borane dimethyl sulfide complex (1.2 mL, 11.84 mmol). The mixture was stirred for 24 h at room temperature until 10 mL methanol was added slowly at 0 °C. The reaction mixture was evaporated under reduced pressure and dried in vacuo for 1 h. The white residue was washed with ethyl ether (3×10 mL) and ethyl acetate (3×5 mL). After washing, the residue was dried in vacuo for 1 h until 20 mL of anhydrous methanol was added. The solution was heated to reflux overnight and then cooled to room temperature. The solution was condensed under reduced pressure and then washed with ethyl ether (3×10 mL). The residue was dried in vacuo to yield the product as a viscous oil (79 mg, 0.06 mmol, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.11 (br, 2H), 6.85 (d, 2H), 6.17 (s, 2H), 4.06 (br, 2H), 3.77 (br, 9H), 3.68–3.55 (m, 14H), 2.65–2.47 (br m, 62H), 1.70–1.62 (br, 28H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 162.1, 160.3, 158.8, 134.1, 130.9, 115.9, 109.2, 91.8, 72.2, 71.9, 71.8, 71.4, 71.0, 67.0, 62.9, 56.5, 56.4, 56.0, 54.2, 53.7, 53.4, 52.9, 41.1, 41.0, 32.0, 30.7, 30.3, 26.2, 24.8, 15.6. HRMS (APCI) calcd for $C_{68}H_{138}N_{15}O_7S$ ($M+H^+$) 1309.0619, observed 1309.0771.

4.4.3. ***HS-TEG-G3CN*** *(**13**).* To a 2-neck 25 mL-round-bottom-flask containing 11 (93 mg, 0.073 mmol) and a stir bar, 9 mL anhydrous dichloromethane was added under argon and the solution was stirred. Triethylsilane (0.3 mL, 3% vol), followed by trifluoroacetic acid (0.5 mL, 5% vol) were then injected to the solution of 24 under argon. The reaction mixture was stirred under argon at room temperature overnight. Evidence of deprotection is displayed by the observation of orange droplets at the surface of the CH_2Cl_2 solution, due to the insolubility of the formed thiol in CH_2Cl_2. After 12 h, the mixture was evaporated in vacuo at room temperature for several hours (to ensure TFA removal). The residue obtained was washed with $CHCl_3$ (3×5 mL) and hexane (2×5 mL) and dried in vacuo to yield 26 (75 mg, 0.068 mmol, 93%) as a pale yellow oil. ^{1}H NMR (CD_3COCD_3): δ (ppm) 7.25 (d, 2H), 6.89 (d, 2H), 4.09 (t, 2H), 3.78 (t, 2H), 3.63–3.56 (br m, 10H), 3.44 (m, 24H), 2.89 (t, 16H), 2.73 (t, 4H), 2.66 (t, 16H), 2.51 (br t, 2H), 2.03 (m, 12H), 1.26 (s, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm) 157.1, 132.9, 129.6, 119.0, 114.6, 72.7, 70.6, 70.5, 70.4, 70.0, 69.5, 67.5, 55.8, 51.9, 51.3, 50.1, 49.9, 48.9, 29.6, 29.2, 23.7, 21.4, 15.9. IR ν_{max} (neat) 2248 cm^{-1} (nitrile). HRMS (ESI) calcd for $C_{58}H_{94}N_{15}O_4S$ ($M+H^+$) 1096.73339, observed 1096.7560.

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Supplementary data

^{1}H NMR spectra and ^{13}C NMR spectra of **G1CN** (**1**), **G1NH$_2$** (**2**), **G2CN** (**3**), **G2NH$_2$** (**4**), **G3CN** (**5**), **TmobS-TEG-OH** (**8**), **TmobS-TEG-OTs** (**9**), **TmobS-TEG-Br** (**10**), **TmobS-TEG-G3CN** (**11**), **TmobS-TEG-G3NH$_2$** (**12**), and **HS-TEG-G3CN** (**13**). ESI spectrum of **HS-TEG-G3CN** (**3**). FTIR of compounds **1**, **2**, **3**, **4**, and **5**. Control experiment on the effect of the presence of trace transition metal on the thiol group of dendron **13**. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.01.070.

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