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Set of Highly Stable Amine- and Carboxylate-Terminated Dendronized Au Nanoparticles with Dense Coating and Nontoxic Mixed-Dendronized Form

Arunendra Saha Ray[†], William E. Ghann^{†,||}, Phoebe S. Tsoi^{†,⊥}, Brian Szychowski[†], Lance T. Dockery[†], Yewon J. Pak^{‡, #}, Wenjing Li[§], Maureen A. Kane[§], Peter Swaan[‡], and Marie-Christine Daniel^{*,†}

[†]Department of Chemistry and Biochemistry, University of Maryland Baltimore County (UMBC), Baltimore, Maryland 21250, United States

[‡]Department of Pharmaceutical Sciences, Center for Nanobiotechnology, University of Maryland, Baltimore, 20 Penn Street, Baltimore, Maryland 21201, United States

[§]Mass Spectrometry Center, University of Maryland School of Pharmacy, Baltimore, Maryland 21201, United States

Abstract

The synthesis of a novel poly(propyleneimine) (PPI) dendron in gram scale as well as its use in the formation of a highly stable, dendronized gold nanoparticle (AuNP)-based drug delivery platform is described herein. The AuNP-based platform is composed of three complementary parts: (i) a 15 nm AuNP core, (ii) a heterofunctional thioctic acid-terminated tetraethylene glycol spacer, and (iii) a third-generation PPI dendron with a unique protonation profile and diverse end-group functionalization that allows for further derivatization. The prepared dendronized AuNPs are able to withstand several rounds of lyophilization cycles with no sign of aggregation, are stable in phosphate-buffered saline and Hanks' buffer as well as in serum, and are resistant to degradation

*Corresponding Author: mdaniel@umbc.edu.

^{||}Center for Nanotechnology, Department of Natural Sciences, Coppin State University, Baltimore, MD 21216, USA.

[⊥]Pharmacology & Chemical Biology, Baylor College of Medicine, Houston, TX, USA.

[#]Food and Drug Administration, Silver Spring, MD 20993, USA.

Author Contributions

A.S.R. synthesized and characterized the dendrons, prepared and characterized the dendronized AuNPs, and carried out most of the cytotoxicity studies and was primarily responsible for preparing the manuscript. W.E.G. designed and prepared the TA-TEG spacer. P.S.T. contributed to the optimization of TA-TEG-OTs spacer synthesis as well as its coupling to the dendron. B.S. prepared the citrate-coated AuNPs and recorded the TEM images. L.T.D. carried out the stability studies of AuNPs with mixed coating. Y.J.P. provided the cell cultures and participated in the cytotoxicity studies. W.L. carried out the MS analysis of dendron 10. M.A.K. supervised the MS data acquisition. P.S. supervised the cytotoxicity studies. M.C.D. conceived the experiments and supervised the synthesized and characterization of the dendrons and of the dendronized AuNPs. A.S.R. and M.C.D. contributed to data interpretation.

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.langmuir.8b03196](https://doi.org/10.1021/acs.langmuir.8b03196). Materials and methods; protocols of the synthesis of spacer intermediates; ¹H NMR spectra and ¹³C NMR spectra of G3CN, TA-TEG-OTs, TA-TEG-G2CN, TA-TEG-G2NH₂, TA-TEG-G3CO₂Me, TA-TEG-G3CO₂H, TA-TEG-G3CN, and TA-TEG-G3NH₂; ESI spectrum of TA-TEG-G2CN, TA-TEG-G3CO₂Me, TA-TEG-G3CO₂H, and TA-TEG-G3CN; additional STEM images and zeta potential data of the dendronized AuNPs; calculation of the maximum theoretical number of TA ligands per AuNP; calculation of the experimental number of dendrons per AuNP, using the TGA data; and stability studies of AuNP-G3CO₂H/NH₂ (PDF)

by glutathione exchange reactions. This nanocarrier platform displays a dense coating, with >1400 dendrons/AuNPs, which will enable very high payload. Furthermore, while amine-terminated AuNPs expectedly showed cytotoxicity against the MCF-7 breast cancer cell line from a NP concentration of 1 nM, the mixed monolayer AuNPs (coated with 40/60 amine/carboxylate dendrons) interestingly did not exhibit any sign of toxicity at concentrations as high as 15 nM, similar to the carboxylate-terminated AuNPs. The described dendronized AuNPs address the current practical need for a stable NP-based drug delivery platform which is scalable and easily conjugable, has long-term stability in solution, and can be conveniently formulated as a powder and redispersed in desired buffer or serum.

Graphical Abstract

1. INTRODUCTION

The current limitations of conventional drug therapy include narrow therapeutic window, systemic toxicity, lack of tissue/organ specificity, and real-time diagnosis.¹ The search for multiplexed molecules that can provide the desired triad of functions (biorecognition, specific cytotoxicity, and biobarrier evasion) is still ongoing and is an area of active research.² However, it is extremely difficult for a single molecule to fulfill all these requirements. One way to simultaneously address these needs is through the judicious selection of nanocarriers³ [liposomes,^{4,5} dendrimers,^{6,7} and nanoparticles (NPs)^{8–12}]. Indeed, because of its size and particular structure, the use of a nanocarrier for the delivery of an existing drug can confer the drug better target specificity (thereby limiting its systemic toxicity), longer circulation time, the ability to overcome some biobarriers, and potential for real-time monitoring.^{10–12}

Currently, multiple pharmaceutical companies have embarked on developing nano-based drug delivery strategies through a careful consideration of the intrinsic challenges and opportunities presented by this nascent technology.² The Food and Drug Administration has in turn approved some of the promising drug nanocarriers such as Doxil (PEG-liposome encapsulation of doxorubicin), Onco TCS (liposomal formulation of vincristine), Abraxane

(albumin-bound paclitaxel NPs), Zevalin (radio-immunoconjugate), and Zinostatin (polymer–protein conjugate).¹³ The success of these drug nanoformulations evidences the potential of this methodology for improving cancer drug therapy.

The use of dendrimers for various drug delivery applications is also an area of intense research.^{6,7,14,15} Dendrimers are highly branched, globular macromolecules with multiple arms emanating from the core.¹⁶ Their well-defined dendritic architecture allows for several advantages over the use of conventional polymers such as (i) controlled multivalence for the linkage of multiple chemical moieties¹⁷ and (ii) very low polydispersity which in turn results in reproducible pharmacokinetic behaviors.¹⁶ Additionally, the drug-loading capacity of the dendrimers can be tuned by varying the generation number (the number of surface groups available for drug interactions doubles or triples with each increasing generation).¹⁶ Likewise, the conjugation of the drug(s) to the dendrimer can be modulated through the nature of chemical linkage (pH-sensitive,¹⁸ light-sensitive,⁴ and biodegradable^{19,20}), and many of these conjugates have shown increased solubility and/or decreased systemic toxicity. Covalent dendrimer–drug conjugates that have been synthesized for ongoing studies have included platinum complexes (cisplatin, oxaliplatin, and carboplatin), 5-fluorouracil, Ara-C, doxorubicin, and paclitaxel.²¹ However, organic core-bearing dendrimers are limited in their sizes because of steric hindrance at higher generations and in turn in the surface area to which molecules of interest might be conjugated.

The creation of nonclassical dendronized NPs, also called NP-cored dendrimers,^{22,23} can circumvent the challenges of increased steric hindrance with higher dendrimer generation by combining a relatively large inorganic gold (Au) core with the defined branched structure of organic dendrons (branched organic macromolecules with a treelike shape).^{24–26} This convergent strategy synthesis also allows for increasing the number of dendritic branches from <10 (in the dendron) to hundreds or thousands (on the dendronized NP, depending on the NP core size) in a single step.^{27,28}

We have previously reported the synthesis of an amine-terminated poly(propyleneimine) (PPI) dendron and its use to prepare stable water-soluble dendronized AuNPs.²⁹ This PPI dendron displayed a protected thiol group at its focal point, the thiol function providing a strong anchor to the AuNP surface and the protective group avoiding thiol interference during further dendron derivatization. While this dendron led to very stable AuNPs, the use of the protective group added two steps to the preparation of the overall dendron (protection/deprotection), with the protection step presenting the poorest yield of the whole synthesis (<50%).

Herein, we report the use of thioctic acid (TA) at the focal point of the PPI dendron, as well as the synthesis of the carboxylate version of the PPI dendron, in addition to the aminated version. This brings significant advantages to both the synthesis and potential derivatization of PPI dendrons as well as the preparation of extremely stable AuNPs, capped with either the carboxylate-PPI dendron, the amine-PPI dendron, or a mixture of both. Indeed, because TA displays a cyclic disulfide, it is not expected to interfere in most common organic reactions, so there is no need of a protective group during the dendron preparation or conjugation: this permits to increase the overall yield of dendron synthesis and to work at

the gram scale. This also extends the choice for further derivatization of the PPI dendrons as there is no more restriction on derivatization conditions because of potential protective group removal and there are no more limitations on the chemistry of derivatization because of potential dendritic label cleavage during deprotection. The use of TA also adds further stability to the dendronized AuNPs because it is a bidentate ligand, as compared to a monodentate thiol group, and endows these reported dendronized AuNPs with high stability both in serum and after multiple freeze–drying cycles.

2. EXPERIMENTAL SECTION

2.1. Synthesis of G2-CN and G3-CN PPI Dendron.

Synthesis was performed following protocols established in our lab and published in prior work.²⁹

2.2. Synthesis of the TA–TEG Spacer.

2.2.1. TA–PFP (1).—TA (10.0 g, 48.5 mmol) was dissolved in 10 mL of dichloromethane (DCM) in a round-bottom flask equipped with a magnetic stir bar. While stirring, *N,N'*-dicyclohexylcarbodiimide (10.0 g, 48.5 mmol) was slowly added, and the mixture was stirred for 15 min. Pentafluorophenol (8.92 g, 48.5 mmol) dissolved in 10 mL of DCM was then slowly added to the mixture and allowed to stir at room temperature (rt) overnight. The mixture was filtered and quenched with 80 mL of water, and the product was extracted with DCM. The combined organic layers were further washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (SiO₂, 9:1 hexane: ethyl acetate) yielded a yellow oil (16.2 g, 90% yield). R_f = 0.85 (9:1 hexane/ethyl acetate); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.62–3.54 (m, 1H), 3.08–3.21 (m, 2H), 2.64–2.73 (t, 2H), 2.42–2.52 (m, 1H), 1.87–1.96 (m, 1H), 1.64–1.86 (m, 4H), 1.46–1.64 (m, 2H). ¹⁹F NMR (376 MHz, CDCl₃): δ (ppm) –152.65 (d, 2F), –157.92 (t, 1F), –162.19 (m, 2F). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 169.37, 142.50, 140.00, 139.90, 138.70, 56.24, 40.29, 38.61, 34.59, 33.21, 28.56, and 24.57.

2.2.2. TA–TEG–OH (5).—TA–PFP (1) (4.50 g, 12.1 mmol), HO–TEG–NH₂ (4) (2.55 g, 13.2 mmol), and triethylamine (1.68 mL, 11.63 mmol) were dissolved in 30 mL of tetrahydrofuran (THF) and stirred overnight at rt. The solvent was evaporated under reduced pressure, and the crude product was purified via column chromatography (10:1 EtOAc/MeOH) to obtain 3.85 g of the product as yellow oil (83% yield); R_f = 0.58 (SiO₂, 10:1 EtOAc/MeOH). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.76–3.38 (m, 17H), 3.20–3.06 (m, 2H), 2.44 (m, 1H), 2.18 (t, 2H), 1.89 (m, 1H), 1.74–1.57 (m, 4H), 1.51–1.35 (2H, m). HRMS (ESI): m/z calcd for C₁₆H₃₂NO₅S₂ (M + H⁺), 382.1716; observed, 382.1714.

2.2.3. TA–TEG–OTs (6).—To TA–TEG–OH (5) (8.0 g, 21 mmol) in 40 mL of THF cooled to 0 °C was added a solution of sodium hydroxide (2.7 g, 67.5 mmol) dissolved in 10 mL of deionized (DI) water, and the solution was stirred for a few minutes. Toluene sulfonyl chloride (12.0 g, 63.0 mmol) in 30 mL of THF was then added slowly using a dropping funnel. The reaction was allowed to stir at rt overnight. The following day, THF was removed under pressure with no additional heat on the rotary evaporator. The clear oil was

then taken up in 100 mL DCM and extracted against water and brine. The combined organic layers were dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified via column chromatography (SiO₂, 95:5 EtOAc/MeOH) to yield 8.7 g of the product as a bright yellow oil (78% yield) R_f = 0.60 (SiO₂, 95:5 EtOAc/MeOH); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.75 (d, 2H), 7.31 (d, 2H), 6.07 (s, 1H) 4.11–4.07 (m, 2H), 3.67–3.35 (m, 16H), 3.08 (m, 2H), 2.38–2.52 (m, 3H), 2.15 (t, 2H), 1.90–1.85 (m, 1H), 1.71–1.51 (m, 4H) 1.51–1.33 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 173.17, 172.91, 145.00, 132.95, 130.00, 129.96, 128.04, 70.50, 60.47, 56.52, 40.30, 39.23, 38.54, 36.39, 34.71, 28.98, 25.45. HRMS (ESI) m/z calcd for C₂₃H₃₇NNaO₇S₃ (M + Na)⁺, 558.1630; observed, 558.1621.

2.3. Synthesis of Spacer–Dendron Conjugates.

2.3.1. TA–TEG–G2CN (7).—G2CN was prepared according to the previously published work.²⁹ To a solution of G2CN (1.3 g, 2.8 mmol) in 10 mL anhydrous CH₃CN, KOH (0.63 g, 11.21 mmol), K₂CO₃ (1.55 g, 11.21 mmol), and tetrabutyl ammonium bromide (TBAB) (87 mg, 0.27 mmol) were added. The reaction mixture was allowed to stir at 45 °C for 2 h in an oil bath after which TA–TEG–OTs (**6**) (3.16 g, 5.88 mmol) was added to the reaction mixture. The final reaction mixture was set to reflux for 2 days. The reaction mixture was then dissolved in 25 mL of DCM and filtered to remove the solids. The organic layer was then poured in DI water and extracted against water and brine. The combined bright orange organic layer was dried over sodium sulfate, filtered, and concentrated to a clear orange oil. The orange oil was subsequently dissolved in a minimum amount of DCM and washed three times with ethyl acetate, Et₂O, and hexane, resulting in the separation of a clear yellow oil (1.8 g, 77% yield). The supernatants collected from the washes were discarded. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.08 (d, 2H), 6.82 (d, 2H), 4.09 (s, 2H), 3.83–3.42 (m, 16H), 3.11 (m, 2H), 2.80–2.48 (m, 31H), 2.15–1.40 (m, 11H). ¹³C NMR (400 MHz, CDCl₃): 173.30, 156.98, 133.04, 129.73, 118.93, 114.47, 77.67, 77.35, 69.79, 56.48, 51.33, 49.61, 45.21, 40.26, 39.18, 36.28, 34.68, 25.43. HRMS (ESI): m/z calcd for C₄₂H₆₆N₈O₅S₂ [M + H]⁺, 827.4670; observed, 827.4699.

2.3.2. TA–TEG–G2NH₂ (8).—To a solution of **7** (1.56 g, 1.89 mmol) in anhydrous THF (40 mL) under nitrogen in a two-neck round-bottom flask was added borane dimethyl sulfide complex (14 mL, 5 equiv per nitrile group, 20 equiv total) using a glass syringe. The reaction mixture was stirred at rt (gel formation was evident on the sides of the flask after few hours). The borane dimethyl sulfide complex was added in two more additions at 4 h intervals. The resulting reaction mixture was allowed to stir overnight at rt under nitrogen. The next day, cold methanol was added slowly at 0 °C until no further bubbling or reaction was observed and was then removed under reduced pressure. Fresh methanol (30 mL) was added to the residue, and the solution was heated under reflux overnight. Upon cooling to rt, the solvent was removed under reduced pressure to yield a light yellow oil. The oily residue was taken up in 20 mL water, washed with ethyl acetate (3 × 10 mL) and diethyl ether (3 × 10 mL), and lyophilized overnight to yield the product as a viscous yellow oil. The oil was dissolved in DI water and further purified through a size exclusion column (LH-20) to yield a yellow oil (1.27 g, 79% yield). Reduction of the nitriles via borane dimethyl sulfide also concurrently reduced the amide bond of TA to an amine, and the completion of this

reduction was observed in the reflected mass spectra. ^1H NMR (400 MHz, CD_3OD): δ (ppm) 7.11 (d, 2H), 6.85 (d, 2H), 4.08 (s, 2H), 3.85–3.36 (m, 16H), 3.12 (m, 2H), 2.95–2.28 (m, 40H), 1.95–1.40 (m, 21H). ^{13}C NMR (400 MHz, CDCl_3) 157.30, 132.71, 129.49, 78.25, 70.44, 48.58, 47.94, 47.73, 47.52, 47.30, 39.72, 34.70, 29.52, 26.80. HRMS (ESI) m/z calcd for $\text{C}_{42}\text{H}_{84}\text{N}_8\text{O}_4\text{S}_2$ $[\text{M} + \text{H}]^+$, 829.6057; observed, 829.6176.

2.3.3. TA–TEG–G3CO₂Me (9).—To a solution of **8** (0.65 g, 0.78 mmol) in 20 mL of MeOH, LiBr (110 mg, 1.25 mmol) was added, and the reaction mixture was stirred for 30 min at 0 °C. Methyl acrylate (3 mL, 5 equiv per amine, 35 mmol) was added in a dropwise manner to the cooled reaction mixture, which was allowed to warm up to rt and further stirred for an additional 48 h. After 48 h, the excess methyl acrylate was removed from the reaction mixture via repeated stripping of the resulting oil with MeOH (4 times), followed by CH_2Cl_2 (4 times). The resulting yellow oil was taken up in 20 mL CH_2Cl_2 and extracted against water and brine. The combined organic layers were then dried over sodium sulfate and filtered to yield a clear light yellow oil (1.1 g, 88%) ^1H NMR (400 MHz, CDCl_3): δ (ppm) (CDCl_3), 7.03 (d, 2H), 6.77 (d, 2H), 4.04 (s, 2H), 3.78–3.38 (m, 43H), 3.12 (m, 2H), 2.85–2.28 (m, 62H), 1.85–1.24 (m, 25H), ^{13}C NMR (400 MHz, CDCl_3): δ (ppm) 173.27, 157.37, 129.54, 114.36, 70.36, 69.61, 67.34, 48.13, 48.92, 31.89, 29.51, 27.13, 23.39. HRMS (ESI) m/z calcd for $\text{C}_{78}\text{H}_{138}\text{N}_8\text{O}_{22}\text{S}_2$ $[\text{M} + \text{H}]^+$, 1603.9445; observed, 1603.9447.

2.3.4. TA–TEG–G3CO₂H (10).—To a solution of **9** (0.5 g, 0.31 mmol) in 10 mL of MeOH/ H_2O (3:1), LiOH· H_2O (42 mg, 6 equiv per ester branch, 16.75 mmol) was added, and the reaction mixture was stirred for 24 h. After 24 h, an additional 15 mL of water was added to the reaction along with 10 mL of CH_2Cl_2 . The extraction against CH_2Cl_2 (10 mL) was performed three times, and the combined aqueous layers were further purified via dialysis [molecular weight cutoff (MWCO) 1000] against pH 7 water. The water was changed four times at an interval of 4–6 h. Post dialysis, the solution was lyophilized, redissolved in DI water, further purified through a size exclusion column (LH-20) in water, and again lyophilized to yield an off-white solid (0.38 g, 83%). Prior to mass spectrometry (MS) acquisition, the product solution was desalted via a PD-10 column. The MS of the sample in 0.1% acetic acid strongly showed the presence of triply charged and doubly charged species at m/z 493.3 $[\text{M} + 3\text{H}]$ and 739.5 $[\text{M} + 2\text{H}]$, respectively. A low-intensity signal for $[\text{M} + \text{H}]^+$ was also observed at 1477.9 via MS. ^1H NMR (400 MHz, CD_3OD): δ (ppm) 7.15 (s, 2H), 6.87 (d, 2H), 4.08 (s, 2H), 3.81–3.54 (m, 16H), 3.11 (m, 2H), 3.12–2.43 (b, 62H), 1.85–1.24 (b, 33H). ^{13}C NMR (400 MHz, CDCl_3) 179.36, 178.55, 157.36, 129.49, 114.35, 70.42, 70.27, 67.69, 52.67, 51.58, 40.04, 38.04, 34.70, 33.83, 29.45, 28.95. ζ potential at pH 7 was –40.6 mV. ATR–FTIR (powder); ν = 3395 O–H stretch, ν = 1725 C=O stretch, ν = 1450 OH bend, ν = 1085 C–O bending; HRMS (ESI) m/z calcd for $\text{C}_{69}\text{H}_{120}\text{N}_8\text{O}_{22}\text{S}_2$: 1476.8 Da; observed mass: 1476.9 Da (both are monoisotopic masses).

2.3.5. TA–TEG–G3CN (11).—G3CN was prepared as previously reported in the literature. G3CN (0.96 g, 1.6 mmol) was dissolved in 10 mL of anhydrous acetonitrile. To the G3CN dendron, 4 equiv of KOH (0.36 g, 6.4 mmol) and K_2CO_3 (0.88 g, 6.4 mmol) were added along with 63 mg (0.19 mmol). TBAB was added and allowed to stir for 1 h in an oil bath at 50 °C. To this mixture, TA–TEG–OTs (**6**) (1.8 g, 4.2 mmol) dissolved in 15 mL

CH₃CN was added and set to reflux for 2 days. After 2 days, the reaction mixture was filtered to remove any solids, and the organic mixture was removed under pressure to yield a viscous orange oil. The orange oil was dissolved in 20 mL of CH₂Cl₂ and extracted twice against water and once against brine. The combined organic layers were dried over sodium sulfate, filtered, and concentrated to a clear orange oil which was dissolved in a minimum of CH₂Cl₂ and washed four times with hexane and diethyl ether to produce a clear orange oil. The supernatant from the washes was discarded (1.48 g, 74% yield). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.05 (d, 2H), 6.78 (d, 2H), 4.05 (s, 2H), 3.78–3.35 (m, 16H), 3.07 (m, 2H), 2.78–2.43 (m, 62H), 2–1.1 (m, 20). ¹³C NMR (400 MHz, CDCl₃) 129.70, 114.60, 70.61, 69.87, 67.50, 49.67, 37.69, 34.24, 29.12. HRMS (ESI) m/z calcd for C₆₆H₁₀₆N₁₆O₅S₂ [M + H]⁺, 1267.7924; observed, 1267.8062.

2.3.6. TA-TEG-G3NH₂ (12).—To a solution of **11** (1.5 g, 1.18 mmol) in anhydrous THF in a two-neck round-bottom flask under nitrogen, the borane dimethyl sulfide complex (4.5 mL, 5 equiv per nitrile branch, 47 mmol) was slowly added using a syringe. The reaction mixture was allowed to stir (gel formation was evident after few hours). Two further additions of the borane dimethyl sulfide complex were performed at 3–4 h intervals. The reaction mixture was allowed to stir overnight at rt under inert conditions. The next day, methanol was added slowly at 0 °C until no further bubbling or reaction was observed. The solvent was then removed under reduced pressure. Fresh methanol (30 mL) was added to the reaction and heated under reflux overnight. Upon cooling to rt, the solvent was removed under reduced pressure to yield a light yellow oil. The oily residue was taken up in 20 mL of water and extracted against ethyl acetate (2 × 10 mL) and diethyl ether (2 × 10 mL). The combined aqueous layers were lyophilized overnight to yield the product as a viscous clear off-white oil. The oil was dissolved in DI water and further purified through a size exclusion column (LH-20), followed by dialysis (MWCO 1000). The dialysis water was changed three times at 4–6 h intervals. Post dialysis, the aqueous solution was lyophilized to yield a very light yellow powder (1.08 g, 71% yield). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.25 (d, 2H), 6.92 (d, 2H), 4.09 (s, 2H), 3.78–3.35 (m, 16H), 3.07 (m, 2H), 3.28–2.43 (m, 78H), 2.26–1.16 (m, 38H). ¹³C NMR (400 MHz, H₂O) 162.88, 130.45, 114.99, 72.82, 70.42, 70.10, 60.67, 40.62, 39.78, 39.78, 39.37, 31.31, 26.7; ATR-FTIR (powder): ν = 3357, 3262 primary amine N–H stretch, ν = 1654 N–H bending. ζ potential at pH 5 was +31.2 mV. HRMS (ESI) m/z calcd for C₆₆H₁₄₀N₁₆O₄S₂ [M + H]⁺, 1286.0685; observed: [M + H]⁺ 1286.0869.

2.4. Synthesis and Studies of AuNPs.

2.4.1. Synthesis of Citrate AuNPs.—All glassware and stir bars were cleaned with aqua regia and rinsed a minimum of 10 times with ultrapure water. A gold salt stock solution was prepared by dissolving HAuCl₄ (0.1 g in 10 mL) in ultrapure water to yield a 28.31 mM stock solution. Sodium citrate stock solution was prepared by dissolving 1 g of sodium citrate in 20 mL of water (0.17 M). A 2.65 mL of gold salt stock solution was added to 247.5 mL of ultrapure water in a two-neck round-bottom flask equipped with a condenser and a stir bar and heated to reflux in an oil bath. Once the solution was refluxed, 4.41 mL of sodium citrate stock solution was added to the reaction mixture and stirred vigorously under reflux. The gold salt to citrate molar ratio used was 1:10. After 20 min, the heat was stopped,

and the solution was kept stirring while cooling down to rt. UV–vis and dynamic light scattering (DLS) spectra were recorded to characterize the AuNPs. The DLS size by number was 16.1 nm.

2.4.2. AuNP Ligand Exchange Reactions.—A 200 mL of citrate AuNPs (2.46 nM) was centrifuged at 10 000g for 60 min at 6 °C to remove some of the excess citrate. The clear supernatant was then removed very carefully and discarded to yield a dark red pellet, which was subsequently dissolved in 30 mL ultrapure water. The absorbance of the citrate AuNP solution at 450 nm was used to calculate the AuNP concentration.³⁰ After the calculation of the citrate-stabilized AuNP concentration, the number of ligands required for complete coverage of the AuNP surface was calculated, and a minimum of 40x the molar amount was used for ligand exchange using both synthesized dendrons (Figure S24).

2.4.3. Singly Dendronized AuNPs.—The amine-terminated dendron (TA–TEG–G3NH₂) was dissolved in 2 mL ultrapure water (pH 5.5), and the carboxylate-terminated dendron (TA–TEG–G3CO₂H) was dissolved in ultrapure water adjusted to pH 9 using NaHCO₃ to ensure complete solubility of respective dendrons. Prior to the addition of the dendron solution to a rapidly stirring solution of AuNPs, the samples were filtered through a 0.22 μ m syringe filter. Upon addition of the dendron solution, the bright reddish AuNP solution turned to a slightly darker red. The solutions were allowed to stir under ambient condition for 20–22 h prior to purification and removal of excess ligands via centrifugation at 20 000g, 45 min at 6 °C (2–3 rounds).

2.4.4. Mixed-Monolayer Dendronized AuNPs.—First, two solutions of amine-terminated dendron (TA–TEG–G3NH₂) and carboxylate-terminated dendron (TA–TEG–G3CO₂H), respectively, were prepared separately by dissolving each in 1 mL ultrapure water adjusted to pH 7 using NaHCO₃. Then the appropriate volumes from each solution were combined to obtain a mixture composed of 60% TA–TEG–G3CO₂H and 40% TA–TEG–G3NH₂ (molar ratio). This mixture was filtered through a 0.22 μ m syringe filter and added to a solution of AuNPs that had been previously centrifuged and redispersed in ultrapure water, as explained for the singly dendronized AuNPs. The AuNP solution turned to a very slightly darker red (if the solution turns purplish, then a drop or two of 1 M HCl should be added). The AuNP solution was allowed to stir under ambient condition for 20–22 h prior to purification and removal of excess ligands via centrifugation at 20 000g, 45 min at 6 °C (2–3 rounds).

2.4.5. Thermogravimetric Analysis.—The dendronized AuNPs were purified extensively prior to the dendron quantification studies via thermogravimetric analysis (TGA). The crude dendronized AuNPs were centrifuged two times for 75 min at 12 000g at 5 °C. After each centrifuge cycle, the colorless supernatant was carefully removed and the remaining dark red pellet was resuspended in ultrapure water. Post centrifugation, the AuNP solution was dialyzed (MWCO: 12 000 Da) three times against ultrapure water. The resulting AuNP solution was centrifuged a third time at 10 000g, 5 °C for 60 min. The supernatant was discarded, and the reddish black pellet was suspended in 15 mL ultrapure water and transferred to a lyophilization chamber. The AuNP samples were lyophilized for 3

days to ensure complete removal of trace amounts of water. The resulting black colored AuNP powders were used for TGA. TGA was run in duplicates for both samples with sample weights ranging from 1.3 to 1.6 mg.

2.4.6. Lyophilization Studies.—The dendronized AuNPs from the AuNP ligand exchange reaction were purified according to the procedure outlined in the TGA sample preparation prior to the dialysis stage. Post centrifugation of the AuNP, the supernatant was discarded and the reddish black pellet was suspended in 20 mL ultrapure water. From the 20 mL batch, a 5 mL aliquot was taken for running UV–vis and DLS data collection. After the acquisition of UV–vis and DLS spectra, the sample was lyophilized for 2 days. The resulting powder was dissolved in exactly the same volume of 5 mL water and was lyophilized. The prior steps of dissolution of AuNPs, data acquisition, and lyophilization were performed for two additional rounds on the same sample, yielding three data points per dendronized AuNP. Care was taken to maintain the same AuNP concentration (2.6 nM) through the three lyophilization rounds.

2.4.7. Stability Studies.—The lyophilized AuNP powders derived as per the procedure in prior steps were dissolved in 2 mL neat solution of buffers and salts with the exception of the serum solution. The concentration for the AuNP used for the stability studies was 1.8 nM. The sample preparation for the study of stability in serum was performed by first dissolving the AuNPs in 250 μ L of ultrapure water to which 1.75 mL human serum was added. All DLS data for stability studies were acquired at 37 °C with an incubation time of 3 minutes. UV–vis data of samples were acquired immediately following DLS data acquisition. The samples were maintained through the entire 7 day period in a water bath maintained at 37 °C.

2.4.8. Evaluation of Cytotoxic Profile of Dendronized AuNPs.—MCF-7 cells were grown in a 250 mL cell culture flask using high-glucose Dulbecco's modified Eagle's medium (DMEM) serum at 37 °C in a humidified atmosphere of 5% CO₂. Cell line was maintained by passaging when cells exhibited 80% confluence. In order to assess the cytotoxic profile of AuNP–G3NH₂, AuNP–G3CO₂H, and AuNP–G3CO₂H/NH₂ (60/40), the respective purified NP formulations were lyophilized overnight to yield a black powder. The AuNP powders were suspended in DMEM via mixing with a sterile 1 mL pipette to generate 15.6 nM initial concentrations. All cytotoxic tests and redispersions were carried out within a BSL-2 laminar flow hood. MCF-7 cells were seeded and dosed according to the protocol outlined above. Cell viability was determined through the equation: cell viability (%) = (sample absorbance at 460 nm – background absorbance at 600 nm)/(absorbance of negative control at 460 nm – background absorbance at 600 nm).

3. RESULTS AND DISCUSSION

3.1. Design and Synthesis of TA–TEG–PPI Dendrons.

In order to ensure the production of a scalable, easily amenable to multifunctionalization, and very stable water-soluble dendronized NP system that can carry very large payloads, we devised a dendron platform composed of three essential components (Figure 1): (1) a PPI

dendritic system which can display either carboxylate or amine termini for convenient further derivatization with desired payload and whose commercial precursors are affordable and readily available from commercial sources; (2) a TEG spacer which allows for maximum coverage of the NP surface by reducing steric hindrance between dendritic branches, and (3) a TA group at the end of the TEG spacer, which imparts very strong AuNP anchoring ability to the dendron.³¹

The use of TA as the anchoring point of the dendron to the AuNPs brings two major advantages over the use of a thiol group: (i) TA provides a stronger attachment to gold surfaces because of the bidentate nature of its terminal cyclic disulfide group^{31–33} and (ii) it eliminates the need for a protective group, thus removing two steps (protection/deprotection) from the synthetic route. This absence of protective group alleviates any concern in the choice of reaction conditions during further derivatization of the dendritic branches.

The PPI dendron platform was obtained by first preparing the TA–TEG spacer and the PPI dendron and then coupling them together. This synthetic approach allowed us to prepare the TA–TEG spacer (Scheme 1) at multigram scale (with each step affording over 85% yield) and the final dendron platform in gram amounts (Schemes 2 and 3).

Two different synthetic routes were used to prepare the amine-terminated PPI dendron and the carboxylate-terminated PPI dendron because of the nature of the chemistry involved in the formation of classical PPI dendron. Indeed, the usual synthetic scheme for the growth of PPI dendrimers or dendrons involves a repetitive sequence of hetero Michael addition using acrylonitrile, followed by reduction of the nitrile groups to primary amines.³⁴ This sequence of steps directly leads to the amine-terminated PPI dendron.

Consequently, for the formation of TA–TEG–G3NH₂, we first prepared the third-generation nitrile-terminated dendron (G3CN) with a phenol group as the focal point, then coupled it to the spacer (TA–TEG–OTs) through nucleophilic substitution at the phenol group, and finally reduced the dendritic nitrile groups to amines using borane dimethyl sulfide. Coupling G3CN instead of G3NH₂ to the spacer avoids any competition of the amine termini with the phenol group, which could result in attachment of the spacer not only to the focal point but also to the dendritic branches. It is interesting to note that the last step, reducing TA–TEG–G3CN into TA–TEG–G3NH₂, concurrently reduces the amide bond in the spacer, between TA and TEG, which is evidenced by the MS data (Figure S5): indeed, the observed mass on the MS data lacks 14 Daltons (16 Daltons less due to the loss of the oxygen of the amide group and 2 Daltons more from the two protons of the reduced amide to secondary amine) compared to the calculated mass for TA–TEG–G3NH₂ containing the amide bond. Other characterization data were collected for TA–TEG–G3NH₂ and are displayed in Figure 2. The ¹H NMR spectrum in D₂O (Figure 2A) shows the peaks from the protons of the dendritic branches spanning from 2 ppm to about 3.3 ppm. The protons from the TEG part give peaks between 3.4 and 4.1 ppm and the TA portion gives rise to peaks mostly between 1 and 2 ppm and also has other peaks hidden within the dendritic peaks between 2 and 3.4 ppm. The aromatic focal point of the dendron is represented by the two peaks at 6.9 and 7.2 ppm. The presence of the terminal primary amine groups is clearly illustrated by the Fourier transform infrared (FTIR) bands at 3357–3262 cm^{–1} (primary amine N–H stretch) and 1654

cm⁻¹ (N–H bending). Finally, the positive zeta potential (30.1 mV) of TA–TEG–G3NH₂ at pH 7 also reflects the presence of the primary amine groups, which are expected to be protonated at this pH.

For the preparation of TA–TEG–G3CO₂H, the last Michael addition needs to involve methyl acrylate in order to lead to carboxylate termini after hydrolysis of the esters. As, on one hand, coupling G3CO₂H to the spacer would not be efficient because of solubility issues as well as potential side reactions and, on the other hand, the dendron–spacer coupling reaction requires the use of bases that would hydrolyze G3CO₂Me during its coupling to the spacer, we decided to couple the spacer to the second-generation nitrileterminated dendron (G2CN). After obtaining TA–TEG–G2CN, we then continued the growth of the dendron through Michael addition using methyl acrylate and obtained TA–TEG–G3CO₂H by hydrolysis using lithium hydroxide.³⁵ Interestingly, following this synthetic path results in the addition of a ninth branch to the final TA–TEG–G3CO₂H. Indeed, in order to maintain the growth of the dendritic branches after coupling to the spacer, TA–TEG–G2CN is reduced to TA–TEG–G2NH₂: during this reduction, the amide bond between TA and TEG also gets reduced into a secondary amine,³⁶ which results in TA–TEG–G2NH₂ displaying nine amines (eight dendritic primary amines and one secondary amine from the spacer). Consequently, the use of an excess (required for the dendritic branches) of methyl acrylate during the following reaction leads to nine –CH₂CH₂CO₂Me branches in TA–TEG–G3CO₂Me. The final hydrolysis results in TA–TEG–G3CO₂H, which contains nine CH₂CH₂CO₂H branches, as evidenced by ¹H NMR integration as well as MS data. The MS data display two prominent peaks: one corresponding to the doubly protonated species (M + 2H) and one for the triply protonated species (M + 3H) (Figure S7). Other characterization data were collected for TA–TEG–G3CO₂H and are displayed in Figure 3. The ¹H NMR spectrum in D₂O (Figure 3A) shows the peaks from the protons of the dendritic branches spanning from 1.6 ppm to about 2.95 ppm. The protons from the TEG part give peaks between 3.5 and 4.1 ppm and the TA portion gives rise to a peak at around 1.35 ppm and also has other peaks hidden within the dendritic peaks between 1.7 and 3.4 ppm. The aromatic focal point of the dendron is represented by the two peaks at 6.8 and 7.1 ppm. The presence of the terminal carboxylate groups is clearly illustrated by the FTIR bands at 3395 cm⁻¹ (O–H stretch), 1725 cm⁻¹ (C=O stretch), and 1450 cm⁻¹ (OH bend). Finally, the negative zeta potential (–40.6 mV) of TA–TEG–G3CO₂H at pH 7 also reflects the presence of the carboxylate groups, which are expected to be deprotonated at this pH.

3.2. Dendronized AuNP Syntheses and Characterization.

3.2.1. Synthesis.—To ensure complete exchange of the citrate molecules with TA–TEG–dendrons on the AuNPs, a minimum of 40 times excess of the TA–TEG–dendron required for total surface coverage was used for each ligand exchange reaction (Figure S24). In addition, we have experimentally determined that the polydispersity indexes (PDIs) of dendronized NPs were not consistent between batches when less than 40 times equivalence of dendrons were used. Furthermore, removal of excess citrate prior to ligand exchange led to lower variability in resulting AuNP polydispersity after ligand exchange across different dendron platforms. It is noteworthy to point out that the presence of excess citrate did not affect the PDI of AuNP–G3CO₂H as much as it did for AuNP–G3NH₂. We hypothesize that

during formation of AuNP–G3NH₂, facile and quick ligand exchange on the Au surface was hindered because of electrostatic attraction between the negatively charged excess citrate and positively charged amine dendrons. On the other hand, steric repulsion between the negatively charged dendron carboxylate and citrate prevented any interference of the excess citrate in the ligand exchange on the AuNPs. Similar findings have been reported on the instability of cationic AuNPs because of the presence of citrate.³⁷ Overall, the removal of excess citrate prior to ligand exchange circumnavigated the discussed difficulties and led to uniformity in the preparation of all batches of dendronized AuNPs.

3.2.2. Characterization.—The purified dendronized AuNPs showed excellent stability in ultrapure water (pH 5.5) (stable >6 months) as a consequence of high steric stability and electrostatic repulsion between highly charged particles as evidenced by zeta potential characterization (Figure 4): in pure water (pH 5.5), the zeta potentials of AuNP–G3CO₂H and AuNP–G3NH₂ are –43.5 and 36.6 mV, respectively. The size of the AuNPs in solution increased from 16.1 nm (DLS by number) for AuNP citrate to 20.4 nm for AuNP–G3CO₂H and from 15.6 to 19.9 nm for AuNP–G3NH₂, which corresponds to an overall increase of 4.3 nm in both cases. This indicates that the hydrodynamic size of dendron coating is 2.15 nm. This corresponds well to the hydrodynamic diameter measured by DLS for the dendron alone (2.3 nm by number).

Dendronized AuNPs with mixed coating (AuNP–G3CO₂H/NH₂, 50/50 molar ratio of TA–TEG–G3CO₂H and TA–TEG–G3NH₂) were also prepared and characterized (Figure 5). The ligand exchange of citrate by the mixture of dendrons led to a surface plasmon resonance (SPR) peak shift of 13 nm (521–534 nm), which is larger than for the singly dendronized AuNPs. Also, the hydrodynamic diameter increased from 17.9 nm (DLS by number) for AuNP citrate to 41.6 nm for AuNP–G3CO₂H/NH₂, which represents an overall size increase of 23.7 nm and is equivalent to a dendritic coating thickness of 11.8 nm. Both the absorption and DLS data seem to indicate the formation of a dendron multilayer around the AuNPs with mixed coating, as opposed to the monolayers formed around singly dendronized AuNPs.

We have also tested AuNP–G3CO₂H/NH₂ (50/50) at different pHs (Figure 5C), and as expected, the AuNPs show an overall negative charge (–32 mV) at pH10 because of the carboxylate groups, a positive charge (30 mV) at pH 5 because of the ammonium groups, and a nearly neutral charge (8 mV) at pH 7, likely coming from equal partial deprotonation and protonation of carboxylate and amine groups, respectively. This evolution of charge reflects well the dendron composition on these mixed-monolayer dendronized AuNPs.

Transmission electron microscopy (TEM) images (Figure 6C) obtained by staining AuNPs with 2% uranyl acetate allowed for visualization of the dendron corona around the NPs, with an average thickness of 1.6 nm. The apparent reduction in dendron thickness calculated from TEM measurements compared to the DLS data (2.15 nm) can be explained by the difference in sample preparation: the DLS measurements are done in solution and at atmospheric pressure, thus the NP coating corresponds to fully hydrated dendrons, whereas the TEM measurements are done on dry samples and under vacuum, thus the NP coating corresponds to contracted dendrons. Further, scanning TEM (STEM) images (Figures S9 and S10) of

concentrated dendronized AuNPs also showed spherical shapes with no apparent aggregation upon visualization.

In order to quantify the extent of surface coating, TGAs were performed. The TGA data indicated that the surface coating of dendrons represents 11.8 and 11.2% of the AuNP masses for AuNP-G3NH₂ and AuNP-G3CO₂H, respectively (Figure 6A,B), which equates to around 1350 and 1450 dendrons/NPs, respectively, calculated via a formula accounting for the core of the NP and molecular weight of the dendron (Figure S25). The lower number of dendrons on AuNP-G3CO₂H can be explained by the presence of nine arms in TA-TEG-G3CO₂H compared to the 8 arms in TA-TEG-G3NH₂ (see the discussion above). The added lateral steric hindrance from the 9th arm leads to a slightly lower dendron packing on the NP surface. However, this slight reduction did not contribute to a lack of NP stability, as showcased in various stability studies (vide infra). The surface density of the dendrons on the AuNPs was calculated to be around 2.3 dendron/nm² for AuNP-G3CO₂H and 2.5 dendron/nm² for AuNP-G3NH₂, which represents a high ligand density but is still realistic as it corroborates well with other observed surface densities.^{38–41}

We also performed some pH studies and noticed that the zeta potential of AuNP-G3NH₂ increases from 36.1 mV at pH 5.5 to 54.3 mV at pH 3 (Figure S10). This increase in positive charges most likely comes from the protonation of the tertiary amines of the dendrons, which we have reported earlier.²⁴ This observation showcases the potential of these dendronized NPs to act as proton sponges at the lysosomal pH of 4.5.

Although other dendrons using TA as the anchoring point²⁵ or also presenting carboxylate or amine termini⁴² have been reported in the literature for the preparation of dendronized NPs, our TA-TEG-G3PPI dendrons bring two main advantages. The first difference is the addition of the TEG linker between the TA group and the PPI dendron itself: this counterbalances the steric hindrance of the eight dendritic branches of each dendron around the gold core, thus allowing very high packing and dense coating on the AuNPs. The other benefit is our preparation of a pair of cationic and anionic PPI dendrons that differ only in the nature of their termini (amine or carboxylate): which allowed us to combine them and prepare the first zwitterionic dendronized AuNPs.

3.3. Stability Studies.

3.3.1. Lyophilization.—In order to test their ease of handling, we investigated the stability of our dendronized NPs against repeated lyophilization cycles. As outlined in Figure 7, both AuNP-G3CO₂H and AuNP-G3NH₂ remained stable even after three rounds of freeze-dry/reconstitution cycles, regardless of the nature of their endgroup functionality: their hydrodynamic diameter showed negligible change after each round. Similar stability was observed for AuNP-G3CO₂H/NH₂ (Figure S26a). We can also note that all of the lyophilized NPs readily redissolved in water, without leaving any precipitate residue. This very high stability is conferred to the NPs not only by the bidentate nature of the TA moiety at the end of the dendritic focal point³³ but also by the high packing density of TA-TEG-dendrons and the steric repulsion between charged dendronized NPs. So far, only very few studies have reported other thiol-protected AuNPs to withstand several rounds of freeze-drying: one featuring cationic ligands,⁴² one using anionic ligands,⁴³ and one with

zwitterionic ligands.⁴⁴ Our present work shows that both TA–TEG–G3CO₂H and AuNP–G3NH₂ lead to very stable anionic and cationic AuNPs, respectively, and also offer the possibility of creating zwitterionic AuNPs starting from nonzwitterionic ligands. These results provide a more versatile solution to the impracticality of storage and long-term handling of NPs in solution. Indeed, because these dendronized NPs are resistant to multiple freeze–dry cycles, they can be stored as powders and reformulated directly into the desired medium (buffers, serum, etc.) right before use. However, they also have the additional advantage of presenting two alternatives for further payload conjugation, through either the carboxylate groups or the primary amine groups.

3.3.2. High Ionic Strength.

Before performing the in vitro cytotoxicity studies (vide infra), we tested the stability of AuNP–G3CO₂H in 1 M NaCl as well as in a more complex salt solution (Hanks' buffer) over the course of a week via DLS (Figure 8). We also evaluated the stability of AuNP–G3CO₂H/NH₂ in 1 M NaCl (Figure S16b). The results clearly demonstrate that both types of NPs exhibit robust stability in the presence of high ionic strength. It is noteworthy to mention that we did observe some NP coating on the glass container in the 1 M NaCl study after 7 days. Nevertheless, this partial deposition of the NPs on glass, due to high ionic concentration, did not cause NP aggregation in the solution dispersion, as reflected in the DLS data (Figure 8). Moreover, AuNP–G3CO₂H did not show any sign of either aggregation or deposition on glass after 7 days in Hanks' buffer (Figure 8A). This observation is an important feature because the intravenous administration (iv) of biopharmaceuticals requires the dissolution of our NPs as a stable nanoformulation at cellular osmolality (100 mM NaCl).^{45,46}

3.3.3. Biologically Relevant Media.—The dendronized AuNPs were tested in glutathione solution and in serum. In order to assess the stability of our NPs against ligand exchange inside cells,⁴⁷ we incubated both AuNP–G3CO₂H and AuNP–G3NH₂ NPs in a 10 mM glutathione solution (the intracellular concentration of glutathione⁴⁸). From the DLS measurements in Figure 9A,B, it is evident that our dendronized AuNP constructs are very stable over a weeklong exposure to 10 mM exogenous glutathione concentration because neither a reduction in size (which would be the result of exchanging the dendrons with glutathione) nor aggregation was observed. Furthermore, our dendronized NPs (AuNP–G3CO₂H) exhibit good stability during incubation in serum at physiological temperature (37 °C): the absorption spectra displayed in Figure 9C show a negligible shift of the SPR peak of the AuNPs, which indicates that no aggregation occurred. The gradual decrease in absorbance observed over time in serum is due to a progressive slow deposition of the AuNPs onto the walls of the glass vial: this is mediated by the serum proteins that have a propensity to adsorb on glass surfaces (most likely facilitated at 37 °C) and can also electrostatically interact with the AuNP ligands. It is noteworthy that the AuNP coating onto glass does stop after 18 h in serum, as indicated by the overlap of absorbance between the measurements at 18 and 24 h in serum. The stability of AuNP–G3CO₂H/NH₂ was also tested in serum, and no sign of aggregation was detected, even after 2 days, as shown by the absence of a red shift of the SPR peak of the AuNPs (Figure S26c).

3.4. In Vitro Cytotoxicity Studies.

Finally, we investigated the toxicity profile of our dendronized NPs as a function of their surface charge. It has been widely reported in the literature that positively charged NPs are toxic because of their detrimental interactions with cell membranes, whereas negatively charged NPs do not generally lead to toxicity.^{49,50} In order to verify the influence of the charge of dendron coating on our AuNPs, we prepared three classes of dendronized NPs and assessed their cytotoxic profile of using MCF-7 cells: (a) AuNP–G3CO₂H, (b) AuNP–G3NH₂, and (c) AuNP–G3CO₂H/NH₂, coated with 60% of TA–TEG–G3CO₂H and 40% of TA–TEG–G3NH₂. From our WST-1 toxicity assay, we observed that indeed the negatively charged AuNP–G3CO₂H NPs were benign up to the NP concentration of 15.6 nM (Figure 10A). The positively charged AuNP–G3NH₂ NPs were cytotoxic at the NP concentration above 1 nM (Figure 10B). However, very interestingly, AuNP–G3CO₂H/NH₂ showed a toxicity profile similar to AuNP–G3CO₂H (Figure 10C) despite the presence of 40% of amino groups on the NP coating that displayed only a slight overall negative charge (–16.8 mV) at pH 7.5 (Figure 10D). This result demonstrates the possibility of safely combining dendrons with various end groups in the formulation of multifunctional dendronized NP constructs.

4. CONCLUSIONS

In conclusion, we have successfully synthesized a dendritic platform that is both versatile and scalable and can be used as coating for the formation of extremely stable AuNPs. The two PPI dendrons prepared herein display either carboxylate or amine termini, which are both easily conjugable functional groups. These can be used either by themselves to create negatively and positively charged AuNPs, respectively, or as a mixed coating to obtain zwitterionic AuNPs. The branched structure of the dendron allows for a higher density of functional groups at the surface of the NPs (up to 1450 dendrons/NPs, equivalent to 11 600 dendritic branches/NPs), further contributing to an increase in the overall stability of the AuNPs. This highly dense but thin dendritic monolayer on the AuNPs will allow for optimum payload of biologically relevant entities while maintaining the size and shape requirements for prolonged circulation time in vivo. In addition, the dendrons can be conjugated to the payload of interest before or after addition to the AuNPs, which further increase the versatility of the system and can lead to the formation of multifunctional AuNPs. Finally, such system could also be used in the construction of other classes of inorganic NPs because of the robust metal–S bonds conferred via the TA anchoring moiety.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Set of PPI dendrons prepared: TA-TEG-G3NH₂ and TA-TEG-G3CO₂H. The sulfur atoms (red) of TA (purple) are the anchoring points for TA-TEG-dendron through Au-S bonds; the role of the tetraethylene glycol (TEG) spacer (blue) is to reduce steric hindrance between arms of third-generation PPI dendrons (black); and the terminal functional groups (brown/green) on PPI dendrons serve as points of conjugation and/or functional group interchange.

Figure 2. Characterization of the TA-TEG-G3NH₂ dendron. (A) ¹H NMR of the TA-TEG-G3NH₂ dendron in D₂O with water suppression. (B) Attenuated total reflection (ATR)-FTIR of TA-TEG-G3NH₂ powder $\nu = 3357, 3262$ primary amine N-H stretch, $\nu = 2928$ C-H unsaturated carbon, $\nu = 1654$ N-H bending, $\nu = 1467$ C-H bend. (C) Zeta potential (ζ) of TA-TEG-G3NH₂ at pH 7.

Figure 3. Characterization of the TA-TEG-G3CO₂H dendron. (A) ¹H NMR of the TA-TEG-G3CO₂H dendron in D₂O with water suppression. (B) ATR-FTIR of TA-TEG-G3CO₂H powder; $\nu = 3395$ O-H stretch, $\nu = 2969$ unsaturated C-H stretch, $\nu = 1725$ C=O stretch, $\nu = 1450$ OH bend, $\nu = 1085$ C-O bending. (C) Zeta potential (ζ) of TA-TEG-G3CO₂H at pH 7.

Figure 4.

(A) Characterization of AuNP-G3CO₂H: (i) UV-vis spectra comparison of citrate AuNPs (blue) vs AuNP-G3CO₂H (green); ligand exchange causes an SPR red shift of 4 nm; (ii) DLS comparison of citrate AuNPs (blue) vs AuNP-G3CO₂H (green) (16.1 nm, PDI 0.05 to 20.4 nm, PDI 0.09); and (iii) zeta potential (ζ) of AuNP-G3CO₂H at pH 5.5. (B) Characterization of AuNP-G3NH₂: (i) UV-vis spectra comparison of citrate AuNPs vs AuNP-G3NH₂ (SPR red shift of 3 nm); (ii) DLS comparison of citrate AuNPs vs AuNP-G3NH₂ (15.6 nm, PDI 0.06–19.9 nm, PDI 0.17); and (iii) zeta potential (ζ) of AuNP-G3NH₂ at pH 5.5.

Figure 5. Characterization of mixed-coated AuNP-G3CO₂H/NH₂ (50/50): (A) UV-vis spectra comparison of citrate AuNPs (blue) vs AuNP-G3CO₂H/NH₂ (orange); (B) DLS comparison of citrate AuNPs (blue) vs AuNP-G3CO₂H/NH₂ (orange); and (C) zeta potential analysis at pH 10 (green), pH 7 (blue), and pH 5 (red)— insets correspond to NP colors at each pH value.

Figure 6. Surface coating of dendronized AuNPs. (A) TGA of dendron coating on AuNP-G3CO₂H. (B) TGA of dendron coating of AuNP-G3NH₂ NPs. (C) TEM image of AuNP-G3CO₂H stained with 2% uranyl acetate for visualization of the dendron corona: average thickness of 1.6 nm.

Figure 7.

Stability of the dendronized AuNPs toward lyophilization. (A) DLS measurements of AuNP-G3CO₂H after three rounds of freeze-dry/dissolution cycles. (B) DLS measurements of AuNP-G3NH₂ after three rounds of freeze-dry/dissolution cycles; inset (i) starting AuNP-G3NH₂ solution, (ii) lyophilized AuNP-G3NH₂ powder, and (iii) AuNP-G3NH₂ solution after resuspension after three rounds of freeze-dry cycles.

Figure 8.

Stability studies of dendronized AuNPs at different ionic strengths. (A) DLS measurements of AuNP-G3CO₂H in Hanks' buffer at time intervals 0, 24 h, 48 h, 5 d, and 7 d. (B) DLS measurements of AuNP-G3CO₂H in 1 M NaCl solution at time intervals 0, 24 h, 48 h, 5 d, and 7 d. Inset: visualization of AuNP-G3CO₂H solution at various time intervals.

Figure 9. Stability study of AuNP-G3CO₂H in biologically relevant media. (A) DLS measurements of AuNP-G3NH₂ in 10 mM glutathione solution at time intervals 0, 1 day, 2 days, and 7 days. (B) DLS measurements of AuNP-G3CO₂H in 10 mM glutathione solution at time intervals 0, 1 day, 2 days, and 7 days; (C) UV-vis spectra of AuNP-G3CO₂H in serum at time points 30 min, 1 h, 2 h, 6 h, 18 h, and 24 h. Inset: visualization of AuNP-G3CO₂H solution in water (control), serum solution, and AuNP-G3CO₂H dissolved in serum after 24 h.

Figure 10.

In vitro cytotoxicity studies of dendronized AuNPs. (A) Toxicity profile of AuNP-G3CO₂H against the MCF-7 breast cancer cell line at NP concentrations ranging from 0.03 to 15.6 nM; (B) toxicity profile of AuNP-G3NH₂ at NP concentrations ranging from 0.03 to 15.6 nM; (C) toxicity profile of AuNP-G3CO₂H/NH₂ (60/40) at concentrations ranging from 0.03 to 15.6 nM; and (D) zeta potential measurements of AuNP-G3CO₂H/NH₂ (60/40) at pH 10 (green), 7.5 (blue), and 5.5 (red).

Scheme 1.

Synthesis of TA-TEG-OTs Spacer from Commercially Available TA and TEG (HO-TEG-OH) Precursors

Scheme 2.
Synthesis Scheme for TA-TEG-G3NH₂

Scheme 3.
Synthesis Scheme of TA-TEG-G3CO₂H