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Esterified Dendritic TAM Radicals with Very High Stability and Enhanced Oxygen Sensitivity

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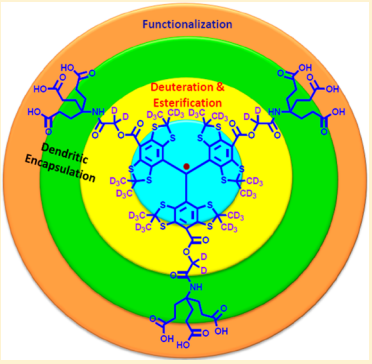
Supporting Information

ABSTRACT: In this work, we have developed a new class of dendritic TAM radicals (TG, TdG, and dTdG) through a convergent method based on the TAM core CT-03 or its deuterated analogue dCT-03 and trifurcated Newkome-type monomer. Among these radicals, dTdG exhibits the best EPR properties with sharpest EPR singlet and highest O_2 sensitivity due to deuteration of both the ester linker groups and the TAM core CT-03. Like the previous dendritic TAM radicals, these new compounds also show extremely high stability toward various reactive species owing to the dendritic encapsulation. The highly charged nature of these molecules resulting from nine carboxylate groups prevents concentration-dependent EPR line broadening at physiological pH. Furthermore, we demonstrate that these TAM radicals can be easily derivatized (e.g., PEGylation) at the nine carboxylate groups and the resulting PEGylated analogue dTdG–PEG completely inhibits the albumin binding, thereby enhancing suitability for in vivo applications. These new dendritic TAM radicals show great potential for in vivo EPR oximetric applications and provide insights on approaches to develop improved and targeted EPR oximetric probes for biomedical applications.



INTRODUCTION

Much effort has focused on developing stable free radicals that are required for a wide variety of applications in functional EPR spectroscopy and imaging (EPRI) as well as Overhauser-enhanced magnetic resonance imaging (OMRI). Among the currently available stable radicals, tetrathiatriarylmethyl (TAM) and nitroxide (NR) radicals represent two major classes of water-soluble paramagnetic probes. TAM radicals have great advantages over NR radicals including high biostability and long relaxation times that provide narrow line width at physiological pH and therefore greatly enhance the sensitivity and resolution for EPRI.[1] In addition, the long relaxation times make TAM radicals easily saturated by radio frequency irradiation affording enhancement of sensitivity and resolution for OMRI with less heating of the sample.[2,3] The long relaxation times of TAM radicals also make them well suited for pulsed EPR/EPRI[4,5] and hyperpolarized C-13 nuclear magnetic resonance and MRI.[6−8]

TAM radicals belong to partially or fully substituted derivatives of triphenylmethyl radical, the first free radical observed in 1900 by Gomberg.[9] Full substitution of each phenyl ring by alkylthio and carboxylate groups effectively eliminates the hyperfine splitting from protons and affords a very sharp EPR singlet. While TAM radicals were first developed by Nycomed Innovation AB as contrast agents for OMRI in the late 1990s,[10−13] these radicals have been structurally modified to expand their applications and so far enable measurement of extracellular[14] and intracellular[15,16] oxygen (O_2) levels, $O_2^{\bullet-}$ generation,[17,18] pH,[19−23] and glutathione levels[24] as well as total redox status.[25,26] Most recently, TAM-based spin labels also have shown great potential for distance measurements in proteins using pulsed EPR dipolar spectroscopy.[27]

High stability and water solubility are two important features of TAM radicals. Current applications of TAM radicals are mostly based on TAM radicals CT-03 and OX063 (Chart 1). However, the stability of both radicals is problematic when exposed to various oxidoreductants. In addition, although CT-03 can be simpler to synthesize than OX063, its relatively high hydrophobicity and tendency for aggregation at high concentrations limits its in vivo application. Therefore, new derivatization strategies for CT-03 are required. Our previous study demonstrated that dendritic encapsulation of CT-03 can effectively enhance the stability of the radical and improve their water solubility under various conditions.[19] Unfortunately, the resulting dendritic TAM radicals DTR1 and DTR2 have relatively broad EPR signal due to the amide linker, resulting in relatively low EPR resolution at a given concentration and low O_2 sensitivity.[19] We have also showed that the esterified derivatives of CT-03 have much simpler and sharper EPR signals owing to the removal of the hyperfine splittings from the amide-*N*.[15] Partial deuteration of the protons in the ester linker can further narrow the EPR lines of TAM radicals.[28] Based on the aforementioned results, we herein developed new dendritic TAM radicals TG, TdG, and dTdG in which the nondeuterated or deuterated dendrons are conjugated to CT-03 or its deuterated analogue dCT-03 through an ester linkage

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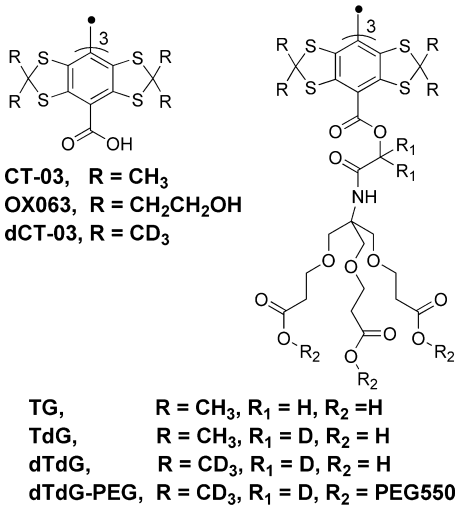
Chart 1. Molecular Structure of Dendritic TAM Radicals[a]



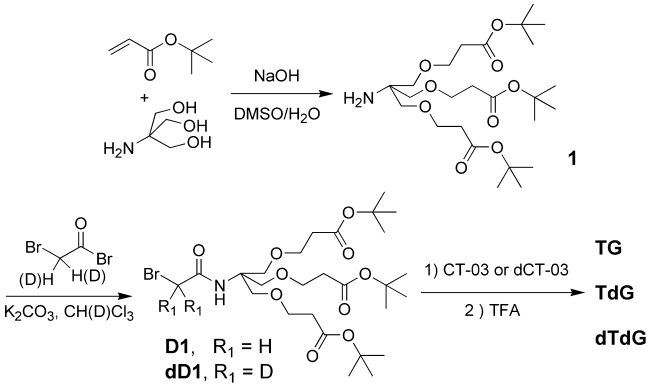
[a]PEG550, poly(ethylene glycol) methyl ether, with average molecular weight of 550. In the nomenclature of new TAM radicals, the letters "T", "d", and "G" mean "TAM radical", "deuteration", and "first generation dendron", respectively. For example, dTdG is an esterified dendritic TAM radical with a deuterated TAM core and the deuterated dendron.

(Chart 1). The O_2 sensitivity and stability toward various oxidoreductants of these radicals were measured, and the concentration-dependence of their EPR spectra was investigated. In addition, in order to further improve its biocompatibility, the PEGylated analogue of dTdG was also developed.

RESULTS AND DISCUSSION

As the first effort, we synthesized the nondeuterated esterified dendritic TAM radical TG using the procedure shown in Scheme 1. The trifurcated Newkome-type monomer **1** was first

Scheme 1. Synthesis of Esterified Dendritic TAM Radicals TG, TdG, and dTdG



obtained by 1,4-addition of tris(hydroxymethyl)aminomethane to *tert*-butyl acrylate according to the reported method.[29] Then, the monomer **1** was reacted with bromoacetyl bromide in the presence of K_2CO_3 to give D1 which was further conjugated with CT-03 to afford TG, followed by deprotection of *tert*-butyl ester groups by TFA. This new convergent procedure improves the synthetic efficiency as compared to the prior divergent method.[19] Attempts to obtain TdG, the deuterated analogue of TG, by directly deuterating the protons in the ester linkers was not successful under different conditions (CF_3COOD/D_2O, K_2CO_3/D_2O–acetone-d_6 (2:1), or K_2CO_3/DMSO-d_6). Therefore, dD1 was synthesized from bromoacetyl bromide-d_2[30] using the same procedure as for D1 and then reacted with CT-03 or dCT-03 to result in TdG and dTdG, respectively.

Figure 1 shows EPR spectra of the newly synthesized esterified dendritic TAM radicals TG, TdG, and dTdG under

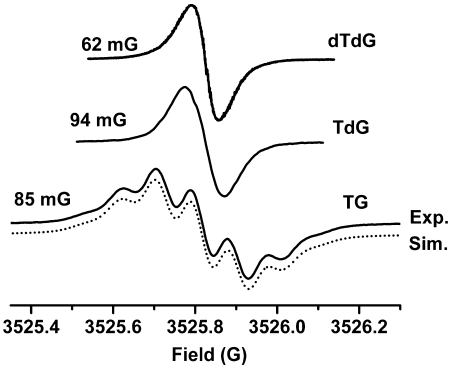


Figure 1. Experimental EPR spectra and linewidths of esterified dendritic TAM radicals under anaerobic conditions. For TG, a simulated spectrum (dotted line) is also shown with the calculated hyperfine splitting of 83 mG from the six protons of the ester linker and calculated line width of 85 mG.

anaerobic conditions. While both TdG and dTdG have an EPR singlet, a multiplet signal was observed for TG with a hyperfine splitting constant of 83 mG due to the interaction of the unpaired electron with six protons from the three ester groups through extensive π conjugation along the central carbon, aryl, and the ester group. As expected, dTdG with a fully deuterated TAM core and ester linkers has the smallest peak-to-peak line width with a value of 62 mG under anaerobic conditions as compared to TG (85 mG) and TdG (94 mG). A slightly broader line width was observed for TdG than TG under anaerobic conditions possibly due to the unresolved hyperfine splittings from the deuterons from the ester groups of TdG.

Owing to its paramagnetic nature, O_2 can undergo Heisenberg spin exchange with exogenous paramagnetic probes in solution, resulting in broadening of the EPR line. The magnitude of this broadening is proportional to the O_2 concentration, which enables the determination of tissue O_2 concentration. High sensitivity of the EPR signal to O_2 is one of the most important properties of TAM radicals for EPR oximetry applications. Figure 2 shows the O_2-dependent line-broadening response of the three TAM radicals. Among these radicals, dTdG has the highest O_2 sensitivity of 3.9 mG/% O_2 with a 130% increase in line width from anaerobic conditions (62 mG) to aerobic conditions (143 mG), while TdG and TG have the O_2 sensitivity of 3.5 and 3.3 mG/% O_2 with approximately 90% and 80% increase in line width, respectively, under the two conditions. Therefore, the use of the fully deuterated TAM core significantly improves the O_2 sensitivity of these radicals. Similar O_2 sensitivity (3.5 mG/%O_2) of TdG with that of the nondeuterated TAM radical CT-03 (3.6 mG/% O_2, Figure 2) indicates that O_2 can still easily diffuse into the dendrimer core of TdG, resulting in similar Heisenberg exchange interactions with TdG as compared to CT-03, although higher generation of TAM dendrimers may have lower O_2 sensitivity due to weaker Heisenberg exchange

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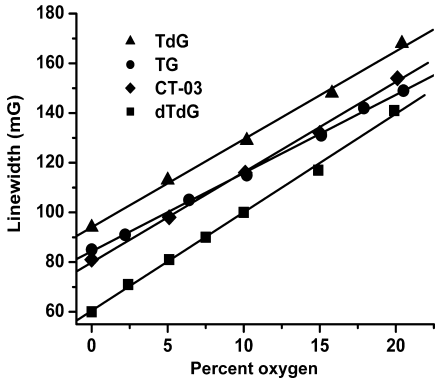

Figure 2. Plots of linewidths of (▲) TdG, (●) TG, (◆) CT-03, and (■) dTdG as a function of O_2 concentration in PBS (pH 7.4).

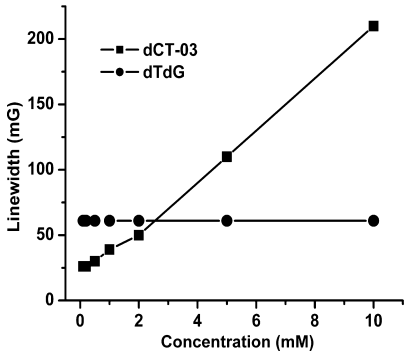

Figure 3. Concentration effect on the linewidths of TAM radicals under anaerobic conditions.

interaction with O_2. On the other hand, these radicals consistently afford much higher response of their EPR linewidths to O_2 than the previous dendritic trityl radicals (<2 mG/% O_2 and 5% line width change) with the amide linker.[19] Moreover, there is >12 times sharper EPR line for dTdG (62 mG) compared to previous dendritic trityl radicals (780 mG) enabling more than 140-fold enhancement of EPR sensitivity.

Despite the relatively high stability of TAM radicals such as CT-03 and OX063, they are still unstable when exposed to reactive species such as superoxide ($O_2^{\bullet-}$)[17−19,31] and peroxidase/hydrogen peroxide (H_2O_2),[32] which limit their in vivo applications. Similar to our previous results,[19] covalent dendritic encapsulation may increase the stability of TAM radicals. Therefore, we herein examined the reactivity of TdG toward oxidants such as hydroxyl radical ($HO^{\bullet}$), peroxyl radical, H_2O_2, peroxynitrite ($ONOO^-$), $O_2^{\bullet-}$, iron(III) (Fe^{3+}), and horse radish peroxidase (HRP)/H_2O_2 as well as reducing agents such as iron(II) (Fe^{2+}), glutathione (GSH), and ascorbic acid. Results showed that TdG has extraordinarily high stability toward all of these highly reactive species with only less than 5% signal decrease after 30-min incubation (Table 1). However, as observed previously,[19,31,32] the stability of CT-03 was problematic, especially when exposed to peroxyl radical, $O_2^{\bullet-}$, peroxynitrite, and HRP/H_2O_2. Cyclic voltammetric studies further revealed that owing to the dendritic encapsulation, esterified dendritic TAM radical TG is resistant to oxidization as evidenced by the absence of peak potential up to 1 V but slightly reducible with a very weak i_{pa} at −0.77 V (see Figure S1, Supporting Information). In comparison, the TAM core CT-03 is redox active as reported in our previous study.[19]

Aside from the enhanced stability, dendritic TAM radicals exhibit much less concentration-dependent line broadening. The intermolecular spin−spin interaction of stable radicals at high concentrations may induce line broadening and thus limit their application for EPR oximetry. As shown in Figure 3, the line width of dTdG did not show any change at concentrations of up to 10 mM which was much lower than the value used for in vivo systems. Comparatively, the concentration of dCT-03 exerts a significant effect on its line width with a value of 18.8 mG/mM when the concentration goes beyond 0.5 mM. Low concentration-dependent line broadening observed for these dendritic radicals could be due to the strong intermolecular static repulsion, resulting from their highly negative charge.

The presence of carboxylate groups in these new esterified dendritic TAM radicals allows further conjugation with various functional molecules to improve their physicochemical properties. It has been shown that albumin binding could broaden the EPR lines of paramagnetic probes due to the shortening of their relaxation times and thus limit their use in EPR oximetry. Covalent attachment of poly(ethylene glycol) (PEG) to these probes may overcome this limitation and also enhance their biocompatibility. Since dTdG has the best EPR properties, it was chosen to be covalently linked with PEG chains. As shown in Scheme 2, the PEGylated TAM radical dTdG-PEG was easily obtained by coupling dTdG with PEG550 (average molecular weight 550) using EDCI in the presence of HOBt and DIPEA.

Scheme 2. Synthesis of the PEGylated Dendritic TAM Radical dTdG-PEG ($n \approx 12$, PEG 550)

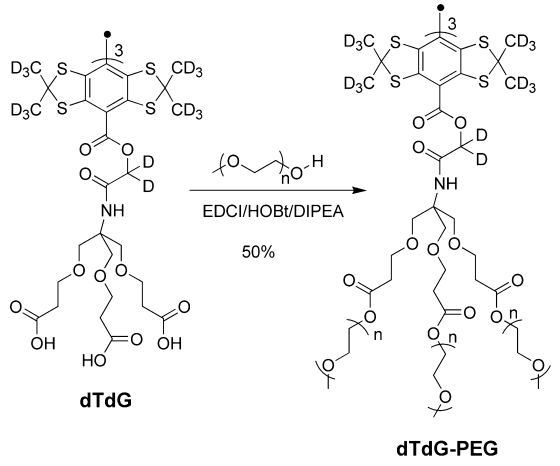

Table 1. Percentage of TAM Radicals Remaining after Exposure to Various Reactive Species in PBS

TAM	Asc	$ONOO^-$	H_2O_2	$HO^{\bullet}$	HRP[a]	$ROO^{\bullet}$	$O_2^{\bullet-}$	GSH	Fe^{2+}	Fe^{3+}
TdG	99	99	100	97	98	97	99	100	99	100
CT-03	97[b]	78	97[b]	91[b]	none	60[b]	55[b]	95[b]	99	99

[a]In the presence of H_2O_2. [b]Data from ref 19. See details in the Experimental Section.

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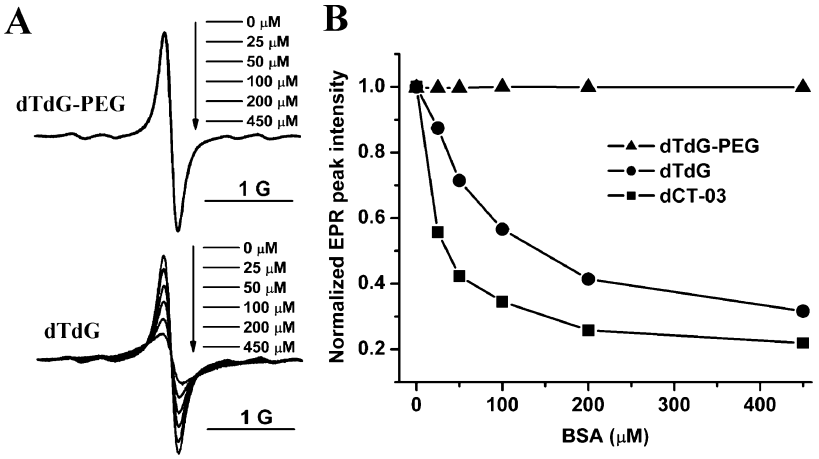

Figure 4. Binding of TAM radicals (20 μM) with BSA. (A) Effect of the BSA concentration on the EPR spectra of dTdG−PEG and dTdG. (B) Plot of the EPR signal intensity of TAM radicals as a function of the BSA concentration.

As expected, the solution of dTdG−PEG (20 μM) in PBS did not show any change in its EPR spectral profile upon addition of BSA (0−450 μM). However, dTdG exhibits binding with BSA as verified by the gradual decrease of its EPR peak intensity as a function of increasing concentration of BSA due to line broadening. The EPR signal double integration of dTdG (20 μM) remains the same before and after addition of BSA (25−450 μM), excluding its spin quenching by BSA (Figure S2, Supporting Information). Compared to dTdG, dCT-03 has even stronger binding with BSA possibly because of the less bulky nature of the latter or due to the relatively higher hydrophobicity of dCT-03, making it easier to interact with BSA than dTdG. As shown in Figure 4, the EPR line width of dTdG−PEG has good response to O_2 with a value of 3.3 mG/% O_2 that is slightly lower than that of dTdG (3.9 mG/% O_2). Of note is that the presence of BSA has no significant influence on the O_2 sensitivity (3.3 mG/% O_2) of dTdG-PEG (Figure 5), supporting its use for in vivo biomedical applications.

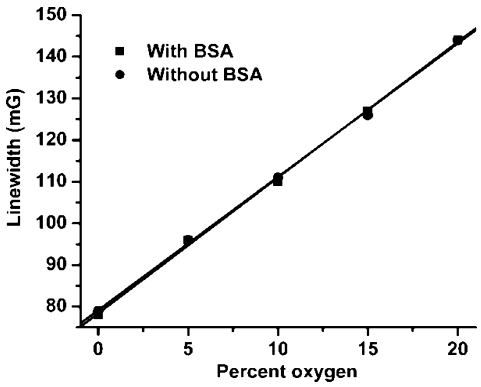

Figure 5. O_2 sensitivity of dTdG−PEG in the presence or absence of BSA (200 μM).

CONCLUSIONS

We have developed a new class of esterified dendritic TAM radicals (TG, TdG, and dTdG) using a convergent method. These new radicals showed extremely high stability toward various reactive species and excellent EPR properties. In particular, the deuterated dTdG has a very sharp EPR singlet which is highly O_2 sensitive and concentration-independent. Importantly, the presence of nine free carboxylic acids in these molecules allows further functionalization to endow new physiochemical properties. As a proof, dTdG was PEGylated on the free carboxylic acid groups, and the resulting TAM radical dTdG−PEG prevents binding to albumin while still maintaining excellent properties for EPR oximetry. Therefore, these dendritic TAM radicals show great potential for EPR oximetric applications and provide a novel strategy for the development of new TAM radicals with various functions.

EXPERIMENTAL SECTION

EPR Measurement. EPR measurements were carried out on Bruker EMX and EleXsys E580 EPR spectrometers at room temperature. General instrument settings were as follows: modulation frequency, 30−100 kHz; microwave frequency, 9.87 GHz; microwave power, 0.2−5 mW; modulation amplitude, 0.01−0.25 G. Measurements were performed in 50 μL capillary tubes.

Oxygen Sensitivity. Oxygen sensitivities of TAM radicals were evaluated according to our previous method. In brief, aqueous solutions of each TAM radical (100 μM) were transferred into gas-permeable Teflon tubes (i.d. = 0.8 mm) that were sealed at both ends. Sealed samples were placed inside a quartz EPR tube with open ends. Nitrogen or N_2/O_2 gas mixtures with varying concentrations of O_2 were allowed to flow into the EPR tube and after at least 10 min equilibration changed to the next gas mixture. EPR spectra were recorded using a model of incremental sweep with the following acquisition parameters: modulation frequency, 30 kHz; microwave power, 0.2 mW; modulation amplitude, 0.01 G.

Stability Studies toward Biological Oxidoreductants. Solutions of GSH (1 mM), Asc (1 mM), and H_2O_2 (1 mM) in PBS buffer (pH 7.4, 50 mM) were used. The Fe(II)−NTA complex was prepared by dissolving $(NH_4)_2Fe(SO_4)_2$ and NTA with a molar ratio of 1:2 in water under anaerobic conditions. The Fe(III)−NTA solution (Fe/NTA 1:2) was prepared by slow addition of the appropriate volume of acidic Fe(III) stock solution into a vigorously stirred solution of NTA in water. The resulting solution was slowly neutralized to pH 7.4 using 0.1 M NaOH. Either Fe(II)−NTA or Fe(III)−NTA solution was freshly prepared before use. Hydroxyl radical ($HO^•$) was continuously generated from the system consisting of Fe(III)−NTA (0.1 mM) and H_2O_2 (1 mM). Superoxide was generated using the xanthine (X)/xanthine oxidase (XO) system using XO (20 mU/mL) and X (0.4 mM) in the presence of DTPA (0.1 mM). Alkylperoxyl radical was generated by thermolysis of 2,2′-azobis-2-methylpropanimidamide, dihydrochloride (AAPH, 1 mM) at 37 °C. Peroxynitrite ($ONOO^-$) was generated by decomposition of SIN-1 (1 mM) at 37 °C in the presence of SOD (50 U/mL). EPR spectra were recorded 30 min after mixing the TAM radical solution (20 μM) with various oxido-

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reductants. Effect of various reactive species on TAM radicals was expressed as percentage of TAM radical remaining after exposure to reactive species for 30 min which was obtained by the double integral of the EPR signal. Each experiment was conducted three times.

Synthesis of D1. To a solution of the trifurcated Newkome-type monomer **1**[29] (3.5 g, 6.9 mmol) in $CHCl_3$ (20 mL) was added solid K_2CO_3 (1.9 g, 13.8 mmol). The resulting suspension was cooled to 0 °C, and bromoacetyl bromide (1.7 g, 8.3 mmol) was added dropwise with stirring over the period of 0.5 h. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was then diluted with 20 mL of $CHCl_3$, and 20 mL of water was later added. The organic layer was washed with 5% citric acid solution (w/v, 20 mL) and then water (20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The resulting residue was purified by column chromatography on silica gel using eluting with hexane/ethyl acetate = 100:5 to 100:10 as eluents to afford D1 as a colorless oil (3.6 g) with a yield of 83%. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.45 (s, $(CH_3)_3C$, 27H); 2.46 (t, CH_2CH_2O, 6H); 3.66 (t, OCH_2CH_2, 6H); 3.71 (s, CCH_2O, 6H); 3.81 (s, $BrCH_2$, 2H); 6.86 (s, NH). ^{13}C NMR ($CDCl_3$, 600 MHz): 28.3 ($(CH_3)_3C$); 29.8 ($BrCH_2$); 36.3 (CH_2CH_2O); 60.4 (CCH_2O); 67.3 (CH_2CH_2O); 69.0 (CCH_2O); 80.7 ($C(CH_3)_3$); 165.9 (CONH); 171.1 (COO-*t*-Bu). HRMS (ESI, $[M + Na]^+$, *m/z*): 648.2352 (D1(Br^{79}), measured), 648.2359 (calcd); 650.2335 (D1(Br^{81}), measured), 650.2339 (calcd).

Synthesis of dD1. The same procedure was applied for the synthesis of D1 using the monomer **1** (1.2 g, 2.4 mmol), K_2CO_3 (0.66 g, 4.8 mmol), bromoacetyl bromide-d_2[30] (0.53 g, 2.6 mmol), and $CDCl_3$ as solvent to afford dD1 as a colorless oil (1.2 g) with a yield of 80%. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.45 (s, $(CH_3)_3C$, 27H); 2.46 (t, CH_2CH_2O, 6H); 3.66 (t, OCH_2CH_2, 6H); 3.71 (s, CCH_2O, 6H); 6.86 (s, NH). ^{13}C NMR ($CDCl_3$, 600 MHz): 28.3 ($(CH_3)_3C$, $BrCD_2$); 36.3 (CH_2CH_2O); 60.3 (CCH_2O); 67.3 (CH_2CH_2O); 69.0 (CCH_2O); 80.7 ($C(CH_3)_3$); 165.8 (CONH); 171.1 (COO-*t*-Bu). HRMS (ESI, $[M + Na]^+$, *m/z*): 650.2577 (dD1(Br^{79}), measured), 650.2483 (calcd); (dD1(Br^{81}), measured), 652.2563 (calcd).

Synthesis of TG. To a solution of CT-03 (80 mg, 80 μmol) and D1 (188 mg, 320 μmol) in DMSO was added solid K_2CO_3 (55 mg, 400 μmol). The reaction mixture was stirred at room temperature for 2 days under N_2 atmosphere. Then, 20 mL of ethyl acetate and 10 mL of 5% citric acid solution (w/v) were added, and the two phases were separated. The organic layer was washed with saturated $NaHCO_3$ solution (10 mL), water (10 mL), and brine (10 mL), dried on anhydrous Na_2SO_4, and concentrated under vacuum. The resulting residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/methanol = 100:0.5 to 100:5 to afford the *tert*-butyl ester-protected form of TG as a brown solid. Thereafter, this brown solid was treated with TFA (2 mL) overnight. TFA was then removed using streaming nitrogen, and the residue was dissolved in phosphate buffer (0.2 M, pH 7.4) and purified by reversed-phase C-18 column chromatography using water followed by 0–20% methanol in water as eluents to give the esterified dendritic TAM radical TG as a brown solid (122 mg, 72%). Purity: 96% by HPLC (see the Supporting Information). ^{1}H NMR (D_2O, 600 MHz): δ 2.39 (br, CH_2CH_2O, 18H); 3.68 (br, OCH_2CH_2 and CCH_2O, 36H). HRMS (MALDI-TOF, $[M]^+$, *m/z*): 2130.161 (measured), 2130.336 (calcd); ($[M + Na]^+$, *m/z*): 2153.181 (measured), 2153.325 (calcd); ($[M + 2Na - H]^+$, *m/z*): 2175.139 (measured), 2175.307 (calcd); ($[M + 3Na - 2H]^+$, *m/z*): 2197.145 (measured), 2197.289 (calcd).

Synthesis of TdG. The same procedure for the synthesis of TG was applied for the synthesis of TdG. CT-03 (50 mg, 50 μmol), dD1 (126 mg, 200 μmol), and K_2CO_3 (35 mg, 250 μmol) in DMSO-d_6 were used to afford *tert*-butyl ester-protected TdG which was further treated with CF_3COOD to afford TdG as a brown solid (85 mg) with a yield of 80%. Purity: > 98% by HPLC (see the Supporting Information). ^{1}H NMR (D_2O, 600 MHz): δ 2.38 (br, CH_2CH_2O, 18H); 3.67 (br, OCH_2CH_2 and CCH_2O, 36H). HRMS (MALDI-TOF, $[M]^+$, *m/z*): 2136.263 (measured), 2136.373 (calcd); ($[M + Na]^+$, *m/z*): 2159.263 (measured), 2159.362 (calcd); ($[M + K]^+$, *m/z*): 2175.256 (measured), 2175.336 (calcd).

Synthesis of dTdG. The same procedure for the synthesis of TG was used for the synthesis of dTdG. dCT-03 (32 mg, 31 μmol), dD1, and K_2CO_3 (17 mg, 120 μmol) in DMSO-d_6 were used to afford *tert*-butyl ester-protected dTdG which was further treated with CF_3COOD to afford dTdG as a brown solid (50 mg) with a yield of 75%. Purity: > 98% by HPLC (see the Supporting Information). ^{1}H NMR (D_2O): δ 2.39 (br, CH_2CH_2O, 18H); 3.68 (br, OCH_2CH_2 and CCH_2O, 36H). HRMS (MALDI-TOF, $[M + H]^+$, *m/z*): 2173.205 (measured), 2173.607 (calcd).

Synthesis of dTdG-PEG. To a solution of dTdG (30 mg, 13.8 μmol), HOBt (83.8 mg, 621 μmol, 45 equiv), and EDCI (120 mg, 621 μmol, 45 equiv) in D_2O (1 mL) and PEG550 (5 mL) was added DIPEA (90 μL) under N_2 atmosphere. The reaction mixture was stirred at room temperature for 3 days, and then 5 mL of 5% citric acid solution was added. The resulting solution was dialyzed against water (3 × 1L) with a molecular weight cutoff of 1000D and further purified by column chromatography on Sephadex G-50 using water as an eluent and then Bio-Beads S-X1 using dichloromethane as an eluent to give the PEGylated TAM radical dTdG-PEG as a brown solid (48 mg, 50%). HPLC: a broad peak at ~2.2 min possibly due to the use of the impure PEG reagent (average MW, 550) and/or the presence of a small fraction of partially PEGylated analogues (see the Supporting Information). ^{1}H NMR (D_2O): δ ~2.5 (br, $C(O)CH_2CH_2$, 18H); 3.23 (s, CH_3O, 27H); 3.48 (br, $CH_2CH_2C(O)$ and CCH_2O, 36H); 3.55 (s, OCH_2CH_2O, ~42SH). HRMS (MALDI-TOF): a broad peak from 6000 to 8000.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Cyclic voltammograms, spectroscopic characterization, and HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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