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Copper-Granule-Catalyzed Microwave-Assisted Click Synthesis of Polyphenol Dendrimers

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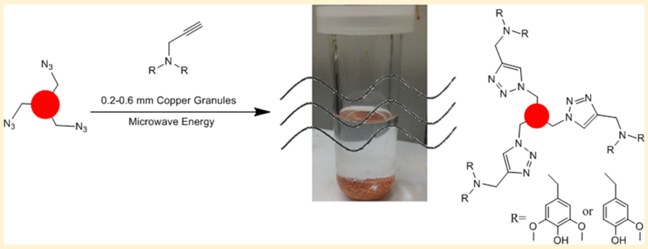
Supporting Information

ABSTRACT: Syringaldehyde- and vanillin-based antioxidant dendrimers were synthesized via microwave-assisted alkyne–azide 1,3-dipolar cycloaddition using copper granules as a catalyst. The use of Cu(I) as a catalyst resulted in copper contaminated dendrimers. To produce copper-free antioxidant dendrimers for biological applications, Cu(I) was substituted with copper granules. Copper granules were ineffective at both room temperature and under reflux conditions (<5% yield). However, they were an excellent catalyst when dendrimer synthesis was performed under microwave irradiation, giving yields up to 94% within 8 h. ICP-mass analysis of the antioxidant dendrimers obtained with this method showed virtually no copper contamination (9 ppm), which was the same as the background level. The synthesized antioxidants, free from copper contamination, demonstrated potent radical scavenging with IC_{50} values of less than 3 μM in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In comparison, dendrimers synthesized from Cu(I)-catalyzed click chemistry showed a high level of copper contamination (4800 ppm) and no detectable antioxidant activity.



INTRODUCTION

Syringaldehyde and vanillin are phenolic aldehydes derived from the breakdown of lignin. Syringaldehyde has various beneficial biological effects, such as antimicrobial activity, antioncogenic effects, and antioxidant activity.[1] Vanillin has been reported to possess antimutagenic, antiangiogenetic, anticolitis, antisickling, and antianalgesic effects.[2] Both of these compounds are weak antioxidants.[3] However, when assembled into dendritic forms, the antioxidant activities are remarkably improved.[4,5]

We previously synthesized antioxidant (AO) dendrimers using "reductive amination"[4,5] However, this synthesis method gave a very low reaction rate and efficiency, especially for dendritic structures with a high number of branches. For example, reactions involving four or more simultaneous reductive aminations gave yields of less than 5% after a two-week reaction.

Cu(I)-catalyzed azide–alkyne cycloaddition, namely, click chemistry, has emerged as one of the most orthogonal and efficient synthesis tools used today. It is especially useful in large molecule synthesis, such as polymer–polymer coupling,[6] antibody–antibody coupling,[7] and in dendrimer synthesis.[8−11] Despite numerous successes, one of the major limitations of click chemistry is copper contamination from the use of Cu(I) as a catalyst. Copper is toxic to certain enzymes. Furthermore, it has been found that the interaction of antioxidants with Cu(I) can trigger cascade radical reactions to form a variety of radicals, resulting in an adverse oxidation process. This deleterious property of an antioxidant is known as the pro-oxidant effect.[4,5,12,13] In our preliminary studies, we have synthesized syringaldehyde- and vanillin-based dendrimers using copper iodide or copper sulfate/sodium ascorbate as the catalyst. However, due to Cu(I) contamination, the antioxidant dendrimers exhibited no antioxidant activities.

To circumvent the use of Cu(I) catalyst, a variety of copper-free click reactions have been developed. These alternative protocols often require high temperature and pressure. In addition, the regioselectivity between 1,4-disubstitution and 1,5-disubstitution is unpredictable.[14,15] Recently, Bertozzi et al. reported the use of cyclooctyne and its derivatives for copper-free, orthogonal click reactions. The reaction was highly efficient even when performed in vivo;[16] the relief of the ring strain is a powerful driving force that links cyclooctyne to azide. This strategy has also been successfully employed in several other fields.[17−21] Another approach that avoids the use of Cu(I) is copper nanoparticles,[22−26] which are often used in combination with a ligand, a base,[26] and/or ultrasound[27,28] to enhance the rate of the reaction. For example, Yus and co-workers reported that, when 10 mol % copper nanoparticles with sizes of 1−5 nm were used in the presence of triethylamine (TEA), nearly quantitative yields were obtained within 30 min.[26] Furthermore, the reaction also demonstrated excellent regioselectivity. However, the nanoparticles with small sizes and, therefore, large surface areas were susceptible to oxidation, especially when the samples were handled for a prolonged period. The oxidized products, CuO and/or Cu_2O were ineffective in the catalysis of the click reaction.[26] In

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comparison, the use of air-stable, large-sized copper metal, such as copper strips or copper granules (1−5 μm), was ineffective, giving no product.[26] The small surface area of these bulky catalysts is likely attributed to the limited alkyne−copper interactions. To use the copper metal (wire) as a catalyst, it had to be used along with ligand(s) or have an already existing triazole ring on the reactant(s), which act as a base or ligand.[29] Weck and co-workers also reported the use of copper wire as a catalyst in dendron synthesis, but the copper metal had to be used along with sodium ascorbate as well as sulfonated bathophenanthroline as a ligand to drive the reaction.[8] In another study, copper metal was used together with $CuSO_4$ for the synthesis of small monomeric species under microwave energy.[30] In the reaction, Cu(I) was generated in situ by comproportionation of Cu(0) and Cu(II). Microwave irradiation helped achieve excellent reaction efficiency.

Microwave energy has been widely employed in organic syntheses to accelerate reaction rates and to improve reaction yields.[31,32] It has also been used to increase the molecular weights in polymer syntheses.[33,34] Under microwave irradiation, polar molecules rapidly change their orientations, allowing functional groups to efficiently interact with larger copper particles. We hypothesize that the use of microwave energy may enable bulky copper granules to effectively catalyze the click synthesis of dendritic antioxidants. Herein, we report a microwave-assisted copper-granule-catalyzed click reaction for the synthesis of syringaldehyde- and vanillin-based dendrimers. The copper level in the synthesized dendrimers and their antioxidant activities were then discussed.

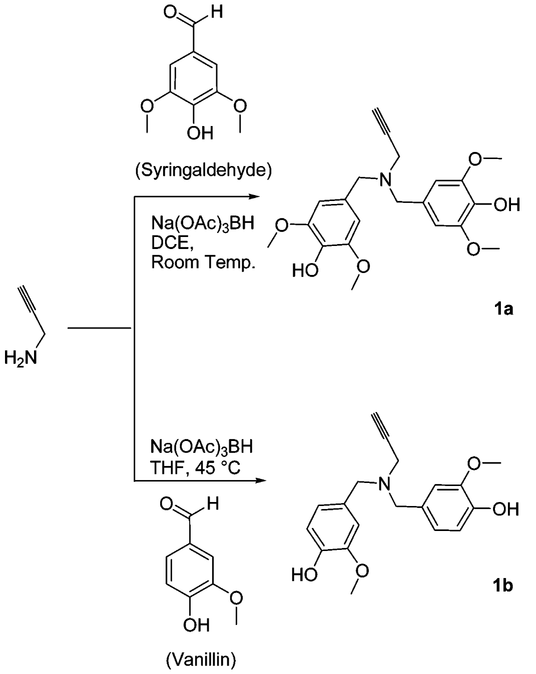
■ RESULTS AND DISCUSSION

Synthesis of Building Blocks and Cores. To synthesize antioxidant dendrimers via alkyne−azide click chemistry, alkyne-derived building blocks **1a** and **1b** were first synthesized through reductive amination of propargylamine with syringaldehyde and vanillin, respectively (Scheme 1; for synthetic details, please see the Supporting Information).

Next, a triazido core (**3**) was synthesized with trimethylolpropane triglycidyl ether (**2**) and sodium azide (Scheme 2). Compound **2** contains three epoxide rings, and each can be opened at two possible positions. Under these reaction conditions, the azide ion only attacked the less-hindered carbons of the epoxide rings, as verified by 2D COSY and HETCOR NMR spectroscopy (Figures S7 and S8 in the Supporting Information). These results are consistent with those reported previously.[35] To test the versatility of the copper-granule-/microwave-assisted click synthesis, a second core (**6**) was synthesized under similar conditions, using 1,3,5-triglycidyl isocyanurate as the starting triepoxide (Scheme 3).

Synthesis of G1 Dendrimers. Using building blocks (**1a** and **1b**) and cores (**3** and **6**), four novel dendrimers (**4a**, **4b**, **7a**, and **7b**) were synthesized via click chemistry (Schemes 2 and 3). The syntheses of dendrimers were initially carried out with either CuI/DIPEA (in anhydrous THF) or $CuSO_4$/sodium ascorbate (in DMF-H_2O, v/v = 9/1). For each method, the amounts of copper catalyst used were 0.3, 0.6, and 3 equiv (0.1, 0.2, and 1 equiv for each branch, respectively). Both systems gave fairly good yields (60−85%). However, the final dendrimers were found to be contaminated with copper ions. Various approaches were attempted to decontaminate the dendrimers, including the use of Sephadex LH-20 (size exclusion chromatography) and 1 M HCl to displace Cu(I) from the dendrimer. Sodium borohydride was also used, aiming to reduce the Cu(I) to Cu(0) to facilitate the decontamination. Unfortunately, none of the methods was successful in complete removal of the copper. The copper ion contamination of an antioxidant can be detrimental to its antioxidant activity. It has been reported that antioxidants can reduce Cu(II) to Cu(I). When these newly formed ions come into contact with O_2 molecules, superoxide radicals are produced,[36−38] which can further react with more antioxidants to produce hydrogen peroxide in the presence of water. Additional Cu(I) ions present will ultimately convert the hydrogen peroxide to hydroxyl radicals, triggering a cascade free radical reaction, consuming the remaining antioxidants. Because of this reason, dendrimers synthesized via Cu(I)-catalyzed click chemistry did not exhibit any antioxidant activities.

Scheme 1. Syntheses of Building Blocks 1a and 1b



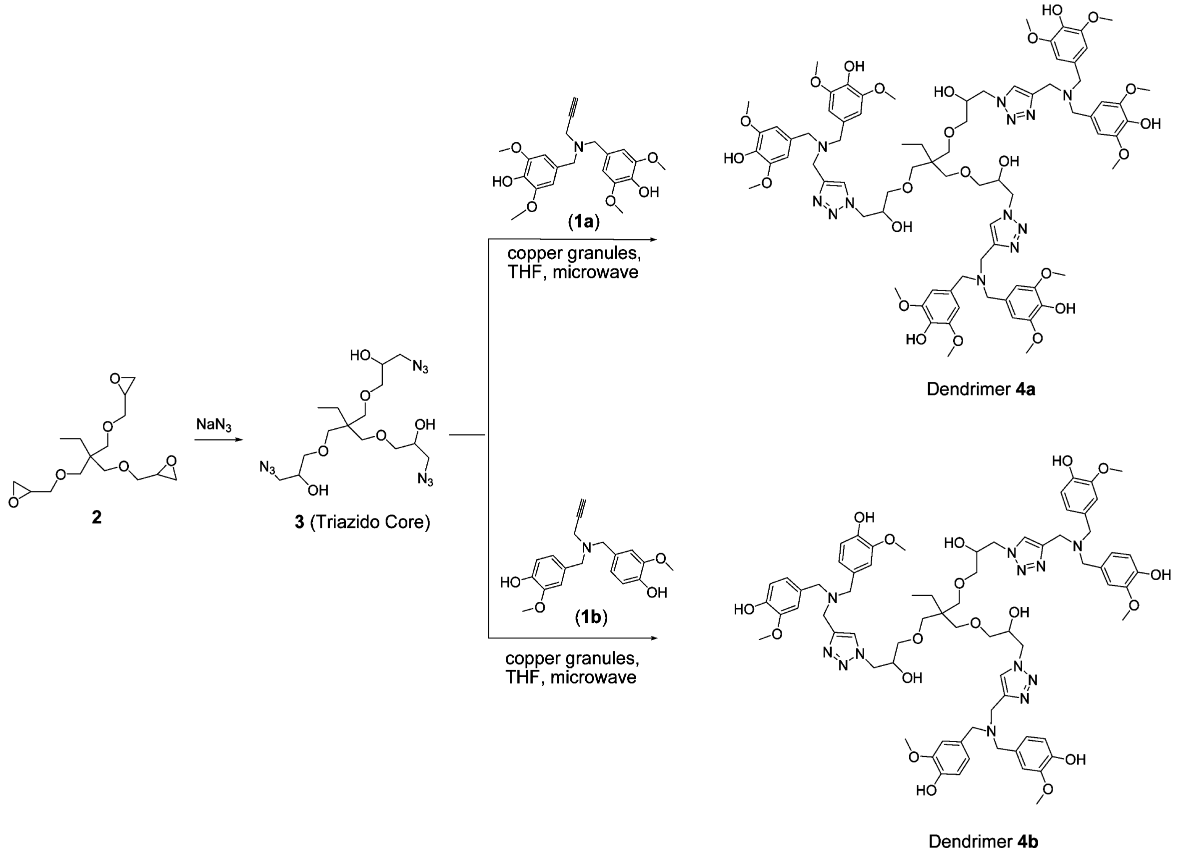
1a

1b

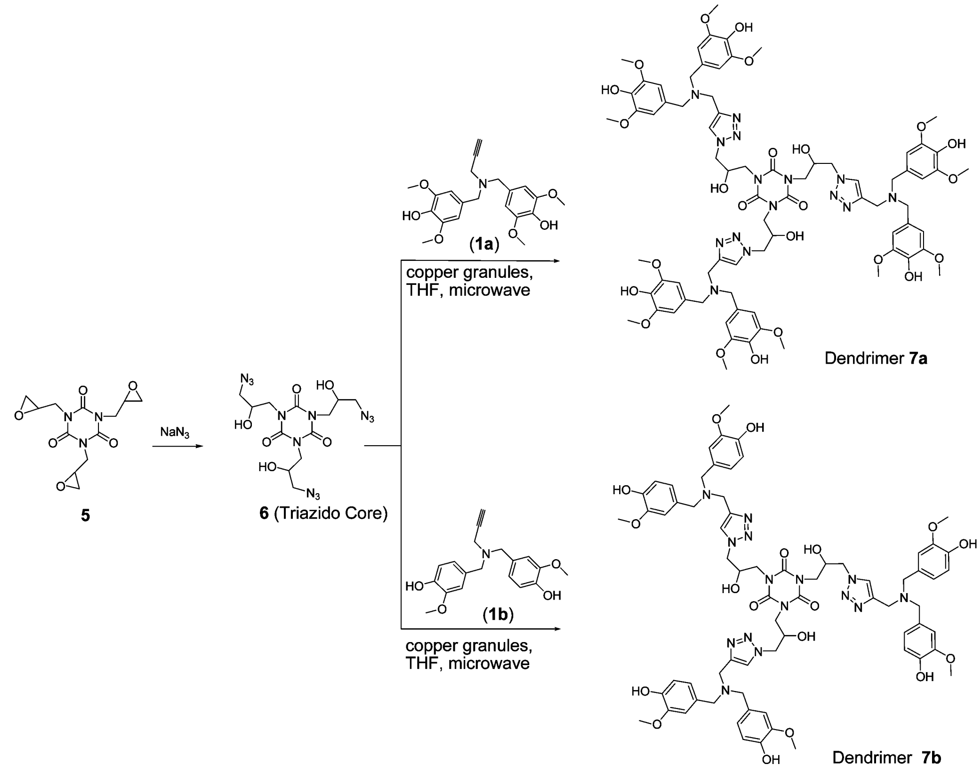
Previous reports showed that the use of copper wire minimizes copper contamination.[29,39] Therefore, we substituted Cu(I) with small copper granules (0.2−0.6 mm diameter) as the catalyst to synthesize our dendrimers. Unfortunately, even at elevated temperature (80 °C), the syntheses with copper granule catalyst only gave poor yields (less than 5%) even after a two-week reaction. The sluggish reaction and the low yields are likely the result of the limited accessibility of the copper catalyst to azide/alkyne functionalities.

Microwave energy has been widely employed to accelerate reactions and to improve reaction yields in small molecule organic syntheses.[31,32] It has also been employed in polymer synthesis to increase molecular weights by improving the accessibility of the reactive functionalities in large polymer molecules.[33,34,40] We hypothesized that, if microwave energy was applied, the reaction rate and efficiency may improve. To test the hypothesis, we first evaluated the reaction between **1a** and **3** (Scheme 2) on a small scale. At low microwave energy (200 W), the reaction was sluggish. Even after reacting for 6 h, there was little product formed, as monitored by UPLC-ESI. The reaction efficiency improved with increasing reaction time: after 7 h, the yield increased to 10%; after 8 h, the yield reached 30% (Figure 1). Encouraged by these results, the microwave

Scheme 2. Syntheses of Dendrimers 4a and 4b



Scheme 3. Syntheses of Dendrimers 7a and 7b



energy was increased to 250 W. The reaction rate was not significantly different during the first 6 h. However, the yields

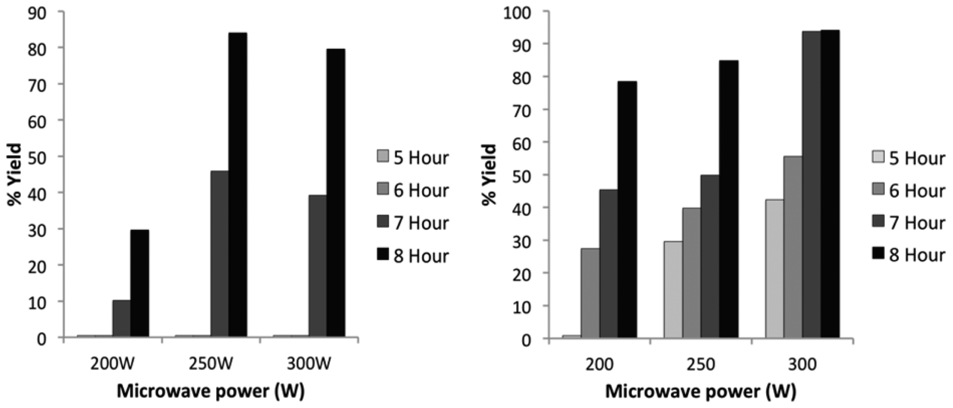


Figure 1. Microwave-assisted synthesis of dendrimer **4a** (left) and **7a** (right) (the percent yields were determined based on UPLC chromatograms, as indicated in the Experimental Section).

Table 1. Reaction Conditions and Yields for Cu-Granule-Catalyzed Click Chemistry

core	building block	product	time (h)	max temp. (°C)	microwave energy (W)	yield[a] (%)
3	**1a**	**4a**	8	77	250	77
3	**1b**	**4b**	8	75	250	78
6	**1a**	**7a**	8	77	250	85
6	**1b**	**7b**	8	73	250	82

[a] Based on isolated yields after silica gel column chromatography purification.

were much better after 7 h (45%) and 8 h (84%) (Figure 1). A further increase in the microwave energy (300 W) did not seem to provide much improvement (Figure 1).

In all cases, there was no product detected by ESI before the first 6 h of reaction. Because of the large number of reactions that occur simultaneously on each molecule, it is not surprising that there is no detectable target product at the early stage of the reaction. Nevertheless, compared to regular heating, this is a significant improvement in the synthesis of complex dendrimers involving multiple alkyne and azide groups. The use of microwave energy thus provides an efficient and convenient approach for macromolecule synthesis. Although the theory behind the microwave effect is controversial, the generally accepted consensus is that, under microwave irradiation, polar molecules rapidly change their orientations according to the microwave frequency. This movement causes higher molecular friction, resulting in faster and more efficient reactions.[33,34] Furthermore, in the synthesis of macromolecules, such as dendrimers and polymers, the reactive functional groups are often wrapped inside the molecules, thereby limiting their accessibility. Irradiation of molecules with microwave energy can rapidly change their orientations and increase the availability of the reactive functionalities, resulting in rapid reaction rates, high yields, and high molecular weight.[40−45] Other factors, such as superheating, and the decrease of bond dissociation energy by increasing polar bond vibration, may also be the major contributions for high efficiency of the microwave reaction.[33,34]

To test the versatility of the copper-granule-catalyzed, microwave-assisted click synthesis, we also evaluated the reaction between **1a** and **6**. As expected, similar results were observed. The maximum yields obtained after 8 h of reaction at 200, 250, and 300 W were 79, 83, and 94%, respectively (Figure 1). It should be noted that, although the reaction under 300 W microwave energy gave a high yield, we observed the color of the reaction solution change to dark brown. UPLC analysis also suggested the formation of byproducts.

On the basis of these results, we concluded that 250 W microwave energy was optimal to "activate" the copper-granule-catalyzed click reaction and avoid side reactions. Subsequent large-scale syntheses of syringaldehyde- and vanillin-based antioxidant dendrimers were performed at 250 W. The results are summarized in Table 1.

The formation of copper-contaminated product is a major dilemma in copper-catalyzed click reactions, especially for the compounds with functional groups that can chelate metal ions and used for biological applications. All of our synthesized dendrimers reported in this study contain many polar functional groups in the core as well as triazole rings that may chelate metal ions. Therefore, we analyzed our purified dendrimers for copper contamination using ICP-MS. The copper impurity presented in the product synthesized via copper-granule-catalyzed microwave reaction was only 9.1 ppm. In comparison, the dendrimer from CuI-catalyzed 'click' synthesis contained a very high level of copper (4800 ppm). As a control, we also measured the copper level in the G1 dendrimer synthesized via reductive amination, which involves no copper catalyst. Surprisingly, the level of copper contamination in this dendrimer was 6.2 ppm. Since no copper was used for its synthesis, this low level of copper may have come from solvents. Indeed, all solvents (hexane, acetone, ethyl acetate, and methanol) contained small amounts of copper (4−31 ppm). On the basis of these results, it can be concluded that the copper-granule-catalyzed microwave-assisted method does not contribute to copper contamination. The reference range for serum copper in humans is about 2 ppm. In pregnant females, it may be as high as 3 ppm.[46] Even if we injected as much as 1 g of our dendrimer (with 9.1 ppm copper contamination) into a human being, the serum copper level will only increase by 0.002 ppm, which is negligible.

Other studies with copper wire as the catalyst showed much higher copper contamination (ca. 40[8] and 450 ppm[29]) compared to our antioxidant dendrimers (9.1 ppm). We are uncertain of this discrepancy. We used copper granules that were prewashed with 25% NaOH, deionized water, 20% H_2SO_4, deionized water, and acetone and were dried with argon. The resulting bright, pinkish copper granules were used immediately after washing. In addition, our reaction was conducted in ultrapure THF (obtained from a solvent purification system) under argon without any ligand or base added. Previous reports suggest that copper metal in the click reaction is an active source of Cu(I).[29] The mechanism by which copper metal is converted to Cu(I) that is required for click chemistry catalysis is still unclear. It has been postulated that there is an initial oxidation of the copper metal surface to Cu(I), which then catalyzes the reaction and, in turn, becomes Cu(II). The Cu(I) ion is regenerated via comproportionation of Cu(0) and Cu(II).[29,47] In another study, sodium ascorbate was used to reduce Cu(II), generated from copper wire, to Cu(I).[8] It should be noted that, although we did not add sodium ascorbate, our reactants (**1a** and **1b**) are also reducing agents that could reduce Cu(II) to Cu(I). We believe that, under our conditions and using prewashed copper granules, only a catalytic amount of copper ions was generated to catalyze the reaction, thereby limiting copper contamination. Nonetheless, the detailed reaction mechanism and low level copper contamination warrants further investigation.

The final synthesized dendrimers (**4a**, **4b**, **7a**, and **7b**) free from Cu(I) contamination were tested for their antioxidant activities with the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. IC_{50} values of dendrimers **4a**, **4b**, **7a**, and **7b** were 2.8, 2.5, 2.2, and 3.0 μM, respectively. In comparison, the Cu(I)-contaminated dendrimers synthesized from Cu(I)-catalyzed click reactions exhibited no detectable antioxidant activities (Table 2). In addition, our novel dendritic antioxidants showed significantly stronger radical scavenging abilities than the naturally occurring and popular antioxidants quercetin (IC50 = 9.0 μM) and vitamin C (IC50 = 16.5 μM), under similar assay conditions.

Table 2. DPPH Assay Results for Dendrimer Antioxidants

antioxidant	IC_{50} (μM)
dendrimer **4a**[a]	2.8
dendrimer **4b**[a]	2.5
dendrimer **7a**[a]	2.2
dendrimer **7b**[a]	3.0
dendrimer **7a**[b]	≫1000
quercetin	9.0
vitamin C	16.5

[a] Synthesized from copper-granule-catalyzed and microwave-assisted click reaction. [b] Synthesized from Cu(I)-catalyzed click reaction.

CONCLUSION

The use of solid copper granules in place of Cu(I) was found to effectively catalyze alkyne−azide click chemistry. Although the catalyst was ineffective under conventional heating, the rates and yields were significantly improved when microwave energy was applied for 8 h. Based on UPLC and NMR analyses, the purity of each final dendrimer (**4a**, **4b**, **7a**, and **7b**) was above 98%. Most importantly, the dendrimers synthesized from this method were virtually copper-free. As a result, these dendrimers exhibited excellent antioxidant activities. Another advantage associated with this synthesis strategy is that no additional ligand or base additives were necessary, thus further simplifying the click reactions.

The use of copper granules in click chemistry coupled with microwave energy offers a simple, convenient, and economical method for the synthesis of large molecules such as dendrimers. The results described herein can provide an important foundation for the syntheses of other antioxidant dendrimers for biological applications using alkyne−azide click chemistry. Thus, the use of copper granules as the catalyst allowed us to solve the long-term problem of product contamination with copper ions in click chemistry.

EXPERIMENTAL SECTION

General Information. Trimethylolpropane triglycidyl ether and 1,3,5-triglycidyl isocyanurate were purchased from commercial sources and were purified by silica gel flash column chromatography prior to use (purification methods were described in detail under compound **3** and compound **6** synthesis).

^1H NMR spectra were recorded at 500 MHz and ^{13}C NMR spectra at 125 MHz. Chemical shifts were reported in parts per million (ppm) with reference to the TMS peak.

ESI mass spectra were obtained using a source capillary voltage of 3000 V, cone voltage of 10 V, and source temperature of 80 °C. Samples analyzed by flow injection had a desolvation gas temperature of 250 °C and a gas flow rate of 200 L/h. The mobile phase was water (50%)−acetonitrile (50%) containing 0.1% formic acid, and its flow rate was 50 μL/min. The injection volume was 10 μL with a sample concentration of approximately 10 ng/μL.

The LC-MS contained a diode array detector at 210−400 nm. A linear gradient from 100% solution A to 80% solution B was used over 4 min at a flow rate of 0.5 mL/min. Solution A consisted of 90% water, 10% acetonitrile, and 0.1% formic acid. Solution B was 100% acetonitrile with 0.1% formic acid. The injection volume was 3 μL with a sample concentration of approximately 10 ng/μL. The conjoined MS unit to the LC had a desolvation gas temperature of 350 °C and a flow rate of 600 L/h. All UPLC samples were filtered through PTFE syringe filters (0.45 μm).

All microwave-assisted reactions were performed using a microwave reactor with predetermined reaction parameters. Reaction temperatures were recorded with an external surface sensor.

The copper granules (0.2−0.6 mm, ≥99.8%) used in the microwave reactions were pretreated in the following order: soaked in 25% NaOH for 20 min, rinsed with deionized water, soaked in 20% H_2SO_4 for 20 min, rinsed with a copious amount of deionized water, rinsed with acetone, dried with argon, and used immediately after.

The copper contaminations were measured using an ICP-MS. The samples (ca. 2 mg) were digested in concentrated HNO_3 (2 mL, ICP grade) for 18 h. The solutions were then diluted to 25 mL using nanopure water. A standard curve was built using a known concentration of copper in 4% HNO_3 (purchased from a commercial source), and the levels of copper in each sample were calculated based on the standard curve.

IC_{50} values for the antioxidants were determined by DPPH reduction as previously reported.[48] The DPPH reagent and all of the antioxidants, except for vitamin C, were prepared in methanol solutions. Vitamin C was in deionized water. The antioxidant sample (25 μL) was added to 1.2 mL of reagent, and the absorbance was measured at 517 nm after 1 h. All samples were run in triplicate at room temperature. The within-run coefficient of variation of the percent inhibition values was less than 6%.

Small-Scale Synthesis to Optimize Microwave Reaction Conditions. ***Dendrimer 4a.*** Triazide core **3** (0.201 g, 0.466 mmol) and building block **1a** (0.574 g, 1.50 mmol) were dissolved in anhydrous THF (25 mL). The solution was then split equally into five 35 mL capacity microwave reaction vessels. Additional anhydrous THF (10 mL) and a stir bar were added to each vessel. Freshly pretreated Cu(0) granules (0.53 g, 8.41 mmol), as mentioned above, were added just before each reaction in the microwave reactor, and the reaction vessel was charged with argon. The microwave reaction was run at varying conditions. Aliquots (100 μL) were removed at predetermined time intervals. The aliquots were filtered through a

PTFE filter (0.45 μm) to remove any particulates, dried, and diluted in a solution of water−acetonitrile (9:1) for analysis on UPLC-ESI. Integrations of UPLC-ESI peaks were compared to a standard curve of known concentrations to calculate reaction yields.

*Dendrimer **7a**.* Triazide core **6** (0.203 g, 0.523 mmol) and building block **1a** (0.640 g, 1.5 mmol) were dissolved in anhydrous THF (25 mL). The solution was then split equally into five 35 mL capacity microwave reaction vessels. Other conditions were the same as those used for dendrimer **4a** optimization.

Standard curves were made with previously synthesized pure compounds (**4a** and **7a**) and analyzed in triplicate. The curves contained three data points of 5, 10, and 17.5 ng/μL and 5, 10, and 15 ng/μL for compounds **4a** and **7a**, respectively.

Large-Scale Synthesis of Antioxidant Dendrimers. *Building Block **1a** (4,4′-((Prop-2-yn-1-ylazanediyl)bis(methylene))bis(2,6-dimethoxyphenol)).* Propargylamine (0.88 g, 16.10 mmol) was added dropwise to syringaldehyde (3.55 g, 19.50 mmol) dissolved in 1,2-dichloroethane (200 mL). The reaction was stirred for 30 min, and then sodium triacetoxyborohydride (3.42 g, 16.14 mmol) was added. The reaction mixture was stirred at rt for 24 h. A second equivalent of syringaldehyde (3.53 g, 19.40 mmol) was added. After 24 h of stirring, a second equivalent of sodium triacetoxyborohydride (3.51 g, 16.56 mmol) was added. After 48 h of stirring, the reaction mixture was washed twice with deionized water. The organic layer was collected and dried with anhydrous $MgSO_4$. After the solution was filtered, the filtrate was condensed under reduced pressure. The resulting residue was redissolved in 5 mL of chloroform and purified by flash column chromatography (with a 40 g prepacked silica gel cartridge). A gradient hexane−ethyl acetate (5:1 → 1:1) solvent system was used as the mobile phase to afford the product as a white crystal (5.5 g, 88%).

R_f = 0.28 (hexane−acetone = 2:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.63 (s, 4H), 5.48 (s, 2H), 3.89 (s, 12H), 3.59 (s, 4H), 3.30 (d, J = 2.4 Hz, 2H), 2.01 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.1, 133.9, 129.9, 105.8, 78.8, 73.7, 57.7, 56.5, 41.4; HPLC: single peak at 18.5 min; HRMS (ESI-TOF) m/z: $[M + H]^+$Calcd for $C_{21}H_{26}NO_6$, 388.1760; found, 388.1757.

*Building Block **1b** (4,4′-((Prop-2-yn-1-ylazanediyl)bis(methylene))bis(2-methoxyphenol)).* Vanillin (3.50 g, 23.02 mmol) was allowed to react with propargylamine (0.80 g, 14.55 mmol) in THF (200 mL) overnight at 40−50 °C. After the reaction was cooled to room temperature, sodium triacetoxyborohydride (3.10 g, 14.62 mmol) was added. From this step and on, heat was no longer used and the reaction was finished with the same method as building block **1a** synthesis.

R_f = 0.52 (hexane−ethyl acetate = 1:1); Yield 57% (2.72 g, light yellow powdery substance); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (s, 2H), 6.87−6.84 (m, 4H), 3.86 (s, 6H), 3.59 (s, 4H), 3.26 (d, J = 2.3 Hz, 2H), 2.17 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 146.4, 144.7, 130.4, 121.9, 114.0, 111.5, 78.5, 73.4, 57.1, 55.8, 40.8; HPLC: single peak at 17.8 min; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{19}H_{22}NO_4$, 328.1549; found, 328.1562.

*Compound **3** (3,3′-((2-((3-Azido-2-hydroxypropoxy)methyl)-2-ethylpropane-1,3-diyl)bis(oxy))bis(1-azidopropan-2-ol)).* Commercially available technical grade trimethylolpropane triglycidyl ether (triepoxide **2**) was purified on a silica gel column. The column was first treated with hexane−ethyl acetate (5:1) containing 2% triethylamine (v/v, 200 mL), followed by hexane (100 mL). Triepoxide **2** was purified on the column using hexane−ethyl acetate as the mobile phase (5:1). The purified compound **2** (R_f = 0.35, hexane−acetone = 3:1) was characterized with GC-MS and ^{1}H/^{13}C NMR and corresponded to literature values.

To compound **2** (10 g, 33.11 mmol) dissolved in DMF (200 mL) were added NaN_3 (7.5 g, 115.38 mmol), NH_4Cl (5.5 g, 102.8 mmol), and deionized water (5 mL). The reaction was run at 50 °C overnight. On completion of the reaction (revealed by the absence of triepoxide **2** on TLC), the reaction mixture was filtered. The filtrate was dried, and the resulting oily substance was resuspended in acetone to further precipitate any remaining solids. The solution was filtered, and the filtrate was condensed under reduced pressure. The resulting crude mixture was purified using silica gel flash column chromatography with a gradient hexane−acetone mobile phase (5/1 → 2/1). The compound **3** was dissolved in methanol (5 mL) and further purified on a 30 g prepacked C18 reverse phase flash chromatography column. The column was eluted with water (50 mL), followed by a CH_3OH−H_2O gradient (2:8, 1 L → 3:7, 1 L) at 15 mL/min. The purity of each fraction was determined by reverse phase HPLC before combining identical ones.

R_f = 0.36 (hexane−acetone = 2:1); Yield 40% (5.74 g, clear colorless oily substance); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.99−3.88 (m, 3H), 3.53−3.30 (m, 18H), 3.09 (s, 3H), 1.40 (q, J = 7.6 Hz, 2H), 0.85 (t, J = 7.6 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 72.6, 72.3, 69.4, 53.3, 43.3, 23.3, 7.6; HPLC: a single peak at 22:01 min; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{15}H_{30}N_9O_6$, 432.2319; found, 432.2324.

*Dendrimer **4a**.* Core **3** (0.12 g, 0.28 mmol), building block **1a** (0.36 g, 0.93 mmol), granulated copper metals (1.59 g, 25.2 mmol), and a stir bar were placed in a 35 mL capacity microwave reaction vessel. The copper granules were pretreated as mentioned above. Ultrapure THF (20 mL) was added to the mixture in the reaction vessel. The reaction vessel was charged with argon, and the microwave reaction was run with conditions set at a maximum temperature of 85 °C, an energy of 250 W, a maximum pressure of 250 psi, and a ramping time of 10 min in power ON mode (reaction is controlled by microwave energy) for 8 h. Final temperatures reached in the reaction vessel were 77 °C at 250 W. The reaction mixture was filtered through Celite, and the filtrate was dried under reduced pressure. The resulting oily substance was resuspended in acetone (5 mL), loaded onto a 40 g prepacked silica gel column, and purified by flash column chromatography. The solvent systems were hexane (100 mL), hexane−ethyl acetate = 1:1 (0.5 L), ethyl acetate (1 L), and ethyl acetate−methanol = 9:1 (2 L). The purification was repeated using the hexane (100 mL) and ethyl acetate−methanol = 9:1 (2 L).

R_f = 0.19 (ethyl acetate−methanol = 8:2); Yield 77% (0.34 g, yellowish amorphous substance); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, J = 1.3 Hz, 3H), 6.61 (s, 12H), 4.56−4.27 (m, 6H), 4.10 (d, J = 2.7 Hz, 3H), 3.82 (s, 36H), 3.72 (s, 6H), 3.52 (s, 12H), 3.42−3.19 (m, 12H), 2.17 (s, 3H), 1.28 (d, J = 7.4 Hz, 2H), 0.77 (t, J = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 146.8, 144.9, 133.5, 129.9, 124.1, 105.4, 72.3, 72.3, 71.5, 68.9, 57.7, 56.1, 52.7, 47.8, 43.2, 22.7, 7.4; HPLC: single peak at 15.74 min; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{78}H_{105}N_{12}O_{24}$, 1593.7365; found, 1593.7345.

*Dendrimer **4b**.* To a solution of triazide core **3** (0.200 g, 0.464 mmol) in dry THF (20 mL) were added building block **1b** (0.520 g, 1.590 mmol), pretreated Cu(0) granules (2.63 g, 41.7 mmol), and a stir bar to a 35 mL capacity microwave reaction vessel. The reaction vessel was flushed with argon, and the microwave reaction was run with conditions set at a maximum temperature of 85 °C, an energy of 250 W, a maximum pressure of 250 psi, and a ramping time of 10 min in power ON mode for 8 h. The reaction mixture was filtered through Celite, and the filtrate was dried under reduced pressure. The reaction mixture dissolved in 5 mL of chloroform was purified by column chromatography with a 40 g prepacked silica gel column. The solvent systems that were used were a hexane−ethyl acetate (1:1, 0.5 L), ethyl acetate (1 L), ethyl acetate−methanol (9:1, 1 L), and finally ethyl acetate−methanol (8:2, 1 L). The purification was repeated using the hexane (100 mL) and ethyl acetate−methanol = 9:1 (2 L).

R_f = 0.46 (ethyl acetate−methanol = 8:2); Yield 78% (0.51 g, light pinkish amorphous substance); ^{1}H NMR (500 MHz, d_6-acetone) δ 7.90 (s, 3H), 7.04 (d, J = 1.7 Hz, 6H), 6.84 (dd, J = 8.0, 1.8 Hz, 6H), 6.78 (d, J = 8.0 Hz, 6H), 4.59 (dd, J = 14.0, 3.4 Hz, 6H), 4.44 (dd, J = 14.0, 7.2 Hz, 6H), 4.19 (s, 3H), 4.05 (q, J = 7.1 Hz, 3H), 3.82 (s, 18H), 3.69 (s, 6H), 3.48 (s, 12H), 3.38 (d, J = 1.7 Hz, 6H), 3.18 (s, 6H), 1.97 (s, 3H), 1.19 (t, J = 7.1 Hz, 2H), 0.85 (t, J = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, d_6-acetone) δ 148.1, 146.3, 145.1, 131.5, 125.2, 122.2, 115.4, 113.0, 73.5, 72.1, 69.8, 57.6, 56.1, 53.7, 48.1, 44.1, 23.4, 8.0; HPLC: 98.9%, major peak at 15.67 min, minor peak at 16.92 min; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{72}H_{93}N_{12}O_{18}$, 1413.6731; found, 1413.6746.

*Compound **6** (1,3,5-Tris(3-azido-2-hydroxypropyl)-1,3,5-triazinane-2,4,6-trione).* 1,3,5-Triglycidyl isocyanurate (triepoxide **5**) is commercially available as technical grade (~70% pure). The compound was purified on a silica gel column prior to use. The column was pretreated with hexane−ethyl acetate (5:1) containing 2% triethylamine (v/v, 200 mL), followed by hexane (100 mL). Hexane−ethyl acetate (5:1) was used as the mobile phase. The purified compound **5** (R_f = 0.66 in hexane−acetone = 1:1) was characterized by GC-MS and ^{1}H/^{13}C NMR and corresponded to literature values.

Synthesis of compound **6** was carried out by reaction of the purified triepoxide **5** (5.5 g, 18.52 mmol) with NaN_3 (4.21 g, 64.77 mmol) and NH_4Cl (3.00 g, 56.08 mmol) in DMF (200 mL). Deionized water (5 mL) was added as a cosolvent, and the reaction was run overnight at room temperature. The reaction mixture was filtered after compound **6** was confirmed by ESI. The filtrate was dried under reduced pressure. The resulting oily substance was resuspended in acetone to further precipitate any remaining salts. After the filtrate was evaporated on a rotovap, it was purified on a silica gel column that was pretreated with a hexane−acetone (4:1) containing 2% triethylamine. The column was then run with a hexane−acetone gradient solvent system (5:1 → 1:1).

R_f = 0.59 (hexane−acetone = 1:1); Yield 55% (4.3 g, clear colorless oily substance); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.24−3.85 (m, 9H), 3.60−3.36 (m, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.1, 68.8, 54.6, 46.4; HPLC: single peak at 14.47 min; HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{12}H_{19}N_{12}O_6$, 427.1551; found, 427.1557.

*Dendrimer **7a**.* Core **6** (0.20 g, 0.47 mmol), building block **1a** (0.56 g, 1.45 mmol), copper metal granules (2.67 g, 42.3 mmol, prewashed as previously mentioned), ultrapure THF (20 mL), and a stir bar were placed into a 35 mL microwave reaction vessel. The vessel was flushed with argon. The microwave reactor was set at an energy of 250 W, a maximum pressure of 250 psi, a maximum temperature of 85 °C, and a ramping time of 10 min. The reaction was run for 8 h in power ON mode. The reaction mixture was then filtered through Celite to remove copper, and the filtrate was condensed under reduced pressure. The remaining residue was redissolved in acetone (5 mL), loaded onto a 40 g prepacked silica gel column, and purified using flash column chromatography. The solvent systems were hexane (0.1 L), hexane−ethyl acetate = 1:1 (0.3 L), ethyl acetate (0.6 L), and ethyl acetate−methanol = 9:1 (2 L). The flow rate was 20 mL/min. The compound was further purified by column chromatography using hexane (0.1 L) and ethyl acetate−methanol = 9:1 (2 L).

R_f = 0.35 (ethyl acetate−methanol = 8:2); Yield 85% (0.63 g, white powdery substance); ^{1}H NMR (500 MHz, acetone-d_6) δ 7.90 (s, 3H), 7.09 (s, 3H), 6.71 (s, 12H), 4.82 (s, 6H), 4.55 (d, J = 10.2 Hz, 3H), 4.36 (d, J = 9.4 Hz, 6H), 4.06−3.83 (m, 6H), 3.78 (s, 36H), 3.67 (s, 6H), 3.48 (s, 12H); ^{13}C NMR (126 MHz, d_6-acetone) δ 148.6, 146.5, 143.3, 133.5, 128.6, 123.2, 104.7, 66.3, 56.0, 54.6, 52.3, 46.2, 45.0; HPLC: single peak at 14.56 min; HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{75}H_{94}N_{15}O_{24}$, 1588.6597; found, 1588.6594.

*Dendrimer **7b**.* Triazide core **6** (0.18 g, 0.423 mmol), building block **1b** (0.43 g, 1.315 mmol), prewashed copper granules (2.40 g, 3.81 mmol), anhydrous THF (20 mL), and a stir bar were added to a 35 mL microwave reaction vessel at room temperature. The reaction vessel was flushed with argon. The microwave reaction was run with power ON mode at a maximum of 85 °C, 250 psi, and 250 W for 8 h. The reaction mixture was then filtered through Celite to remove copper, and the filtrate was removed under reduced pressure. The resulting residue was redissolved in acetone (5 mL), loaded onto a 40 g prepacked silica gel column, and purified by flash column chromatography. The solvent systems were hexane (0.2 L), hexane−ethyl acetate = 1:1 (0.5 L), ethyl acetate (1 L), and ethyl acetate−MeOH = 9:1 (1 L). The compound was further purified by the column chromatography using hexane (0.1 L) and ethyl acetate−methanol = 9:1 (2 L).

R_f= 0.35 (ethyl acetate−methanol = 8:2); Yield 82% (0.49 g, white powdery substance); ^{1}H NMR (500 MHz, acetone-d_6) δ 7.91 (s, 3H), 7.52 (s, 3H), 7.04 (d, J = 1.3 Hz, 6H), 6.87−6.80 (m, 6H), 6.76 (d, J = 8.0 Hz, 6H), 4.85 (s, 6H), 4.59 (d, J = 10.3 Hz, 3H), 4.38 (d, J = 9.2 Hz, 6H), 3.98 (ddd, J = 18.6, 13.6, 5.9 Hz, 6H), 3.82 (s, 18H), 3.66 (s, 6H), 3.47 (s, 12H); ^{13}C NMR (126 MHz, acetone-d_6) δ 150.6, 150.6, 148.2, 146.3, 145.3, 131.6, 125.2, 122.2, 115.4, 113.0, 68.4, 57.6, 56.1, 54.3, 48.1, 47.0; HPLC: 98.5%, major peak at 14.56 min, two minor peaks at 14.01 and 16.81 min; HRMS (ESI-TOF) *m/z*: $[M + H^+]$ Calcd for $C_{69}H_{82}N_{15}O_{18}$, 1408.5962; found, 1408.5952.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H/^{13}C NMR spectroscopy data for all of the synthesized compounds. 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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