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# Strain-Promoted Alkyne Azide Cycloaddition for the Functionalization of Poly(amide)-Based Dendrons and Dendrimers

Cátia Ornelas, Johannes Broichhagen, and Marcus Weck*

*Molecular Design Institute and Department of Chemistry, New York University, New York, New York 10003-6688*

Received December 15, 2009; E-mail: marcus.weck@nyu.edu

***Abstract:*** Functionalization of a poly(amido)-based dendron with ethylene glycol chains (PEG) using copper-catalyzed alkyne azide cycloaddition (CuAAC) afforded dendrons with significant levels of copper contaminations, preventing the use of such materials for biological applications. We suggest that the presence of amide, PEG, and triazole functional groups allows for copper complexation, thereby preventing the separation of the copper catalyst from the final dendron. To minimize this problem, synthetic variations on CuAAC including the addition of "click" additives for copper sequestering as well as the use of copper wire as the copper source were investigated. None of these strategies, however, resulted in copper-free products. In contrast, we developed a copper-free strain-promoted alkyne azide cycloaddition (SPAAC) strategy that functionalized poly(amide)-based dendrons and dendrimers with PEG chains quantitatively under mild reaction conditions without any metal contamination. The SPAAC products were characterized by $^{1}$H and $^{13}$C NMR, 2D HSQC and COSY NMR, mass spectrometry, and elemental analysis. This is the first report on the use of SPAAC for dendrimer functionalization, and the results obtained here show that SPAAC is an important tool to the dendrimer and more general biomaterials community for the functionalization of macromolecular structures due to the mild and metal-free reaction conditions, no side products, tolerance toward functional groups, and high yields.

## Introduction

Over the past decades, a variety of drug delivery carriers including polymer microcapsules, liposomes, and nanoparticles have been explored for biomedical applications such as drug delivery, gene delivery, and in vivo imaging.[1−7] Among these materials, dendrimers have emerged as important candidates for these applications.[8−16] In comparison to linear polymers, dendrimers have a number of advantages including: (i) the controlled multivalency of dendrimers that can be used to attach several drug molecules, targeting groups and solubilizing moieties; (ii) the low polydispersity, which provides reproducible pharmacokinetic behavior; and (iii) the globular shape of higher generation dendrimers, which has the potential to affect their biological properties.[17]

Since the first iteration of a Michael addition followed by nitrile reduction demonstrated by Vögtle in 1979,[18] functionalization of dendrimers was successfully achieved using this[19] and other synthetic methodologies including amide and ester couplings,[20] Williamson reactions,[21,22] and cross-metathesis.[23−25]

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None of these reactions, however, can be carried out easily in vitro or in vivo. Furthermore, in some cases, nonquantitative functionalization steps as well as functional group compatibility have limited the success of these strategies. One synthetic methodology that has the potential to overcome these limitations is the use of "click chemistry", which was introduced by Sharpless and co-workers in 2001.[26] Click reactions have to meet several criteria including high yields, tolerance toward functional groups, and virtually no side-reactions. Among the "click" reactions, the Cu(I)-catalyzed alkyne azide 1,3-dipolar cycloaddition (CuAAC) is the most popular.[27] CuAAC was successfully applied in different areas of materials chemistry[28] including, but not limited to, polymers,[29] nanoparticles,[30] interlocked molecules,[31−33] and dendrimers.[34] In dendrimer chemistry, CuAAC was used for the convergent[35] and divergent[36,37] syntheses of dendrimers as well as in their functionalization.[34,38−43] The cytotoxicity of copper, however, is a potential drawback of the CuAAC reaction for the synthesis and/or functionalization of nanomaterials such as dendrimers for biomedical applications. In cases where the final structure contains numerous functional groups able to bind copper ions, the removal of the copper catalyst can be problematic, limiting the use of copper-contaminated drug delivery carriers for pharmaceutical purposes.[44,45] Therefore, copper-free "click" strategies that combine the advantages of CuAAC without the use of a toxic transition metal would be highly desirable for biomedical applications.[44]

Recently, an interesting copper-free reaction based on strained cyclooctynes and azides was developed by Bertozzi and co-workers for in vivo applications.[46−48] This reaction goes back to the late work of Georg Wittig who described the exothermic cycloaddition of cyclooctyne with phenyl azide leading to the corresponding triazole.[49] The Bertozzi group has applied this strain-promoted alkyne azide cycloaddition (SPAAC) in covalent modifications of living systems[50] and in vivo imaging of membrane-associated glycans in developing Zebrafish using a multicolor detection strategy.[51] This reaction was then applied by other groups to label peptides[52] and lipids,[53] to cross-linked hydrogels,[54] photodegradable star polymers,[55] and to functionalize polymers.[56,57]

In this contribution, we describe the limitation of copper-based 1,3-dipolar cycloaddition functionalization strategies in poly(amide)-based dendrons and present SPAAC as an efficient copper-free strategy to functionalize dendrons and dendrimers. We utilize poly(amido)-based dendrons that are known to be biocompatible and therefore are an interesting starting point for novel biomaterials. Furthermore, they can be multifunctionalized through the use of either bifunctional dendrons[40] or a trifunctional core,[58] bringing us closer to our long-term goal, the construction of well-defined dendrimers for theranostics that require several functionalities including (i) hydrophilic groups such as PEG chains to increase water solubility and biocompatibility, (ii) imaging agents to monitor the trajectory of the dendrimer in vitro or/and in vivo, (iii) targeting groups such as folic acid, biotin, or specific antibodies, and (iv) the desired drugs. Herein, we report the use of SPAAC as a highly advantageous and efficient functionalization strategy toward multifunctional dendrimers.

## Results and Discussion

**Synthesis of Poly(amide)-Based Dendrons and Dendrimers with Azide Termini.** The key to the development of a 1,3-dipolar cycloaddition-based functionalization strategy in dendrimer chemistry is the synthesis of the azide-containing dendrons and dendrimers.[59] We employed poly(amide)-based dendrons that follow the 1→3 connectivity pioneered by Newkome.[60] Dendrons containing three and nine azide termini and a dendrimer containing 18 azide termini were synthesized in high yields from commercial dendrons nitrotriester **1** and aminotriester **5** as shown in Scheme 1.

Our strategy is based on building block **1**. The deprotection of the acid groups on **1** was carried out by stirring **1** in formic acid at room temperature for 24 h, resulting in dendron triacid


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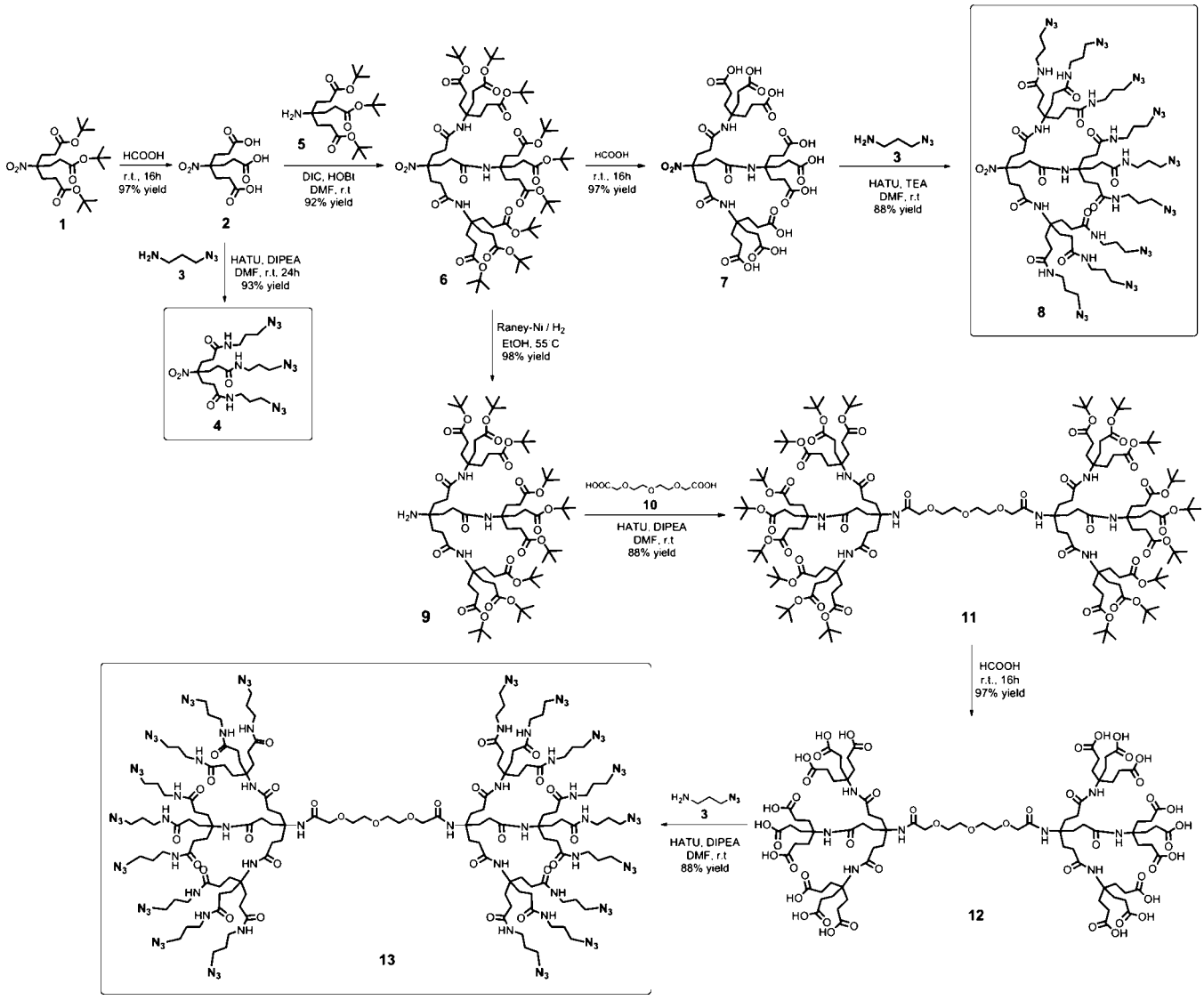
***Scheme 1.*** Synthesis of Dendrons Tris-azide **4** and Nona-azide **8**, and Dendrimer Eighteen-azide **13**




**2**.[60,61] Dendron tris azide **4** was synthesized by the coupling reaction between the acid groups on **2** and 3-aminopropyl azide **3** using HATU as the coupling agent. After purification by column chromatography, **4** was obtained as a colorless oil in 93% yield (for NMR and mass spectra of **4**, see the Supporting Information). Peptide coupling between **2** and **5** afforded dendron nitro-nonaester **6** that after deprotection of the acid termini using formic acid yielded dendron nitro-nonaacid **7**. The coupling reaction between the acid groups on **7** and **3** afforded dendron nonaazide **8** that after purification by column chromatography was obtained as a colorless oil in 87% yield. The $^1$H NMR and $^{13}$C NMR spectra of **8** show all of the expected signals including the C$H_2$N$_3$ at 3.36 ppm, the C$H_2$CH$_2$CH$_2$N$_3$ at 3.24 ppm, and the $C$H$_2$N$_3$ at 50.2 ppm, NO$_2$$C_q$ at 94.5 ppm, and NH$C$═O at 175.8 and 173.7 ppm. The typical band for azide groups at 2098.30 cm$^{-1}$ was observed by IR spectroscopy, and the mass spectrum shows the molecular ion peak of **8** $(M + Na)^+$ at 1732.9 (calcd for $C_{67}H_{114}N_{40}O_{14}Na$: 1725.9) (for $^1$H NMR and mass spectra of dendrons **6**, **7**, and **8**, see the Supporting Information).

Reduction of the nitro group on **6** was carried out at 45 psi $H_2$ and 55 °C in the presence of Raney-Ni yielding dendron amino-nonaester **9** as a white powder in 89% yield.[61,62] The coupling reaction between the amine group at the focal point of **9** and the acid groups of the diacid-PEG linker **10** afforded dendrimer **11** that was purified by dialysis against methanol using a MWCO (molecular weight cutoff) 2000 dialysis membrane. Dendrimer **11** was obtained as a colorless waxy product in 85% yield. The $^1$H NMR spectrum shows all of the expected signals including the OC$H_2$CONH group of the PEG linker at 3.97 ppm, suggesting the completion of the coupling of the dendrons to the linker (no signal was observed for free C$H_2$COOH). The MALDI-TOF mass spectrum of **11** shows the molecular ion peak $(M + H)^+$ at 3070.2 *m*/*z* (calcd for $C_{160}H_{278}N_8O_{47}$: 3065.0). Deprotection of the acid termini on **11** was achieved by stirring in formic acid at room temperature for 48 h. Completion of the deprotection reaction was verified by $^1$H NMR spectroscopy by the disappearance of the COO(C$H_3$)$_3$ signal at 1.45 ppm. The mass spectrum of the resulting dendrimer **12** containing 18 acid termini shows the

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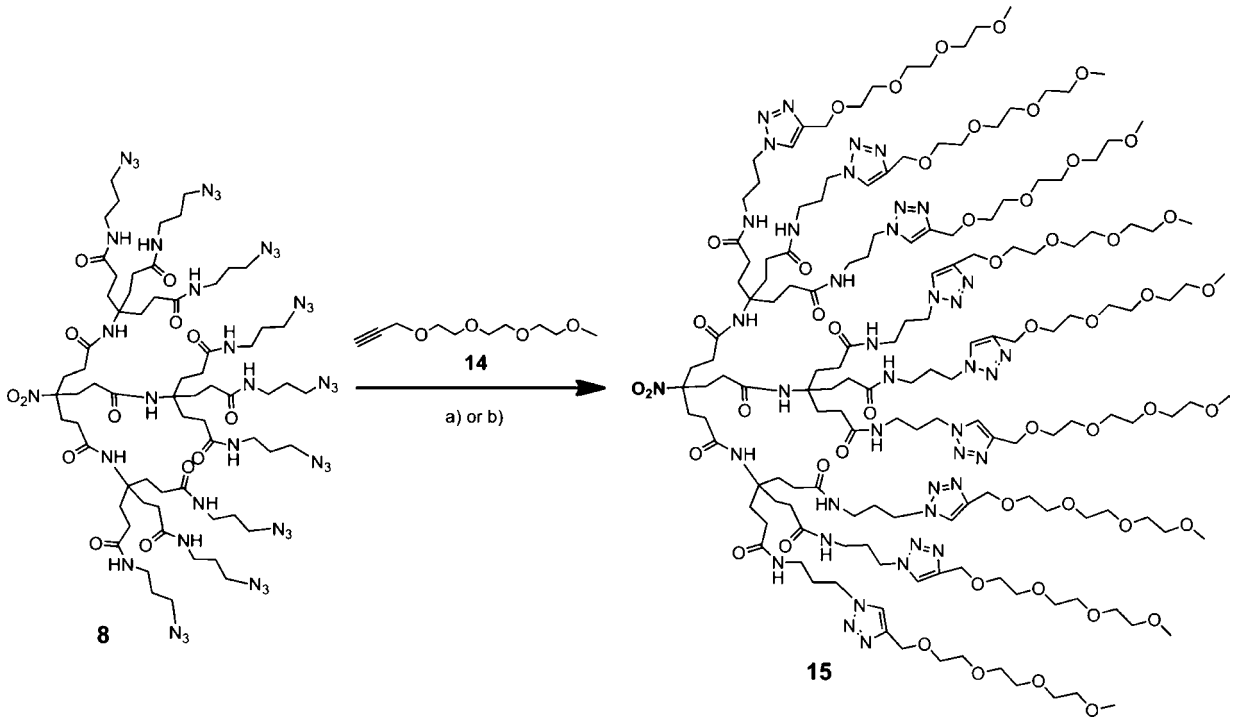
***Scheme 2.*** CuAAC between Dendron **8** and Alkyne Derivative **14**[a]




[a] (a) $CH_2Cl_2/H_2O$ (1:1), $CuSO_4 \cdot 5H_2O$, sodium ascorbate (SA), room temperature, 24 h; (b) $CH_2Cl_2/H_2O$/EtOH (1:1:1), CuI, sulfonated bathophenanthroline (SBP), sodium ascorbate, room temperature, 24 h.

molecular ion peak $(M + Na)^+$ at 2078.5 (calcd for $C_{88}H_{134}N_8O_{47}Na$: 2077.8). The azide termini were then introduced into the dendrimer by the coupling reaction between the acid termini on **12** and **3** affording dendrimer eighteen-azide **13**. After purification by dialysis against methanol using a MWCO 2000 dialysis membrane, **13** was obtained as a colorless oil in 76% yield. The $^1H$ NMR spectrum of **13** shows all of the expected peaks including the signals that correspond to the PEG linker ($OCH_2CONH$ at 3.91 ppm and $OCH_2CH_2O$ at 3.65 ppm) and the triplet that corresponds to the $CH_2N_3$ group at 3.35 ppm. The IR spectrum shows a band at 2098.30 $cm^{-1}$, confirming the presence of azide groups.

**Functionalization of Poly(amide)-Based Dendrons and Dendrimers with PEG Chains Using CuAAC.** To obtain water-soluble dendrons, we carried out the functionalization of **8** with alkyne derivative **14** that contains a PEG chain, which often endows materials with water solubility, biocompatibility, and avoids attachment to nonspecific proteins. We first investigated the functionalization of **8** using CuAAC (Scheme 2).

Our first choice for the CuAAC reaction (method A) was a mixture of $CH_2Cl_2/H_2O$ (1:1) as solvent and $CuSO_4$/sodium ascorbate (SA) as the Cu(I) source, and the reaction was stirred at room temperature for 24 h. To remove the copper catalyst from the target dendron **15**, we investigated several workup procedures typical for "click" dendrimers including extraction,[35,41] chromatographic methods,[63,64] and dialysis.[65]

To extract dendron **15** from the reaction mixture, an aqueous solution of ammonium hydroxide was added followed by the extraction of the reaction with methylene chloride. Analysis of the organic and aqueous phases showed that dendron **15** remained in the aqueous phase together with the copper catalyst and sodium ascorbate. The aqueous phase was evaporated to dryness, redissolved in methanol, and filtered. After evaporation of the solvent, a light green waxy product was obtained. The green coloration suggested contamination of dendron **15** with copper, making this workup procedure not usable.

Purification of **15** by column chromatography was attempted using silica gel ($SiO_2$) and C18-silica gel reverse-phase column chromatography. In both cases, a green layer remained on top of the columns, suggesting the removal of a significant amount of copper. The products obtained, however, also had a light green coloration, still suggesting the contamination with copper.

We also tried to purify **15** by dialysis against water and against an aqueous solution of ammonium hydroxide or EDTA. Again, the green coloration of the product inside the dialysis bag remained (for detailed information about the workup procedures, see the Experimental Section).

Despite our efforts to remove the copper catalyst from **15**, a green waxy product was obtained for all workup procedures, suggesting the contamination with copper. CHN elemental analysis of **15** synthesized by method A ($CuSO_4$/SA) confirms the nonpurity of the final product (Anal. Calcd for $C_{157}H_{276}N_{40}O_{50}$: C, 53.51; H, 7.89; N, 15.90. Found: C, 44.54; H, 6.75; N, 9.77). The lower amount of carbon in the elemental analysis suggests the presence of inorganic impurities. To confirm the presence of copper, ICP-MS analysis of **15** was performed, and 0.50% of copper was found.

To minimize the copper contamination on **15**, we varied the CuAAC reaction conditions. Several reports in the literature suggest that in the CuAAC reaction so-called "click additives" can be used to stabilize the Cu(I) and even accelerate the reaction

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***Table 1.*** Reaction Conditions Used in CuAAC between Dendron **4** and Alkyne Derivative **14** Using Copper Wire as the Copper Source[a]

| | reducing agent[b] | ligand[c] | solution color | reaction time (h) | reaction conversion[d] |
|---|---|---|---|---|---|
| 1 | | | blue/greenish | 24 | 0% |
| 2 | SA | | brown | 24 | 0% |
| 3 | | PMDETA | yellow | 24 | 0% |
| 4 | SA | PMDETA | yellow | 48 | 0% |
| 5 | | SBP | yellow | 24 | 0% |
| 6 | SA | SBP | dark red | 48 | 100% |

[a] Reactions were carried out in $^t$BuOH/$H_2O$ (1:1) at room temperature. [b] The amount of sodium ascorbate (SA) added was 40 mol % per mol of azide groups. [c] The amount of ligand added was 20 mol % per mol of azide groups. [d] The reaction conversions were obtained by $^1$H NMR spectroscopy after workup.

kinetics.[66−69] In particular, sulfonated bathophenanthroline (SBP) was shown to be a very useful additive because it enhances significantly the reaction rate and it is efficient on copper sequestering precluding protein degradation caused by Cu(I).[70] Therefore, we investigated the synthesis of **15** using different reaction conditions that include SBP. The CuAAC reaction was carried out using a previously prepared "click" solution[32] that consisted of a deep red water/ethanol (1:1) solution of CuI, SA, and SBP (method B). The "click" solution was added to a methylene chloride solution of **8** and **14**, and the mixture was stirred at room temperature for 24 h. However, all attempts to isolate **15** from the "click" solution including the above-described extraction, column chromatography, reverse-phase column chromatography, and dialysis methods resulted in a green waxy product, also suggesting the presence of copper. CHN elemental analysis of **15** synthesized by method B also confirms the nonpurity of the final product (Anal. Calcd for $C_{157}H_{276}N_{40}O_{50}$: C, 53.51; H, 7.89; N, 15.90. Found: C, 40.92; H, 6.11; N, 9.57). The contamination of **15** with copper was also confirmed by ICP-MS analysis, showing a copper content of 0.007% in the final dendron. This result demonstrates that the ability of SBP to sequester copper decreased the amount of copper contamination, but it did not result in complete removal of copper from **15**.

In the literature, several reports suggest that the use of copper wire as the Cu(I) source minimizes copper contamination in CuAAC reaction.[71−73] Therefore, we investigated the use of copper wire for the CuAAC reaction between the poly(amide)-based dendrons and **14**. Because the use of Cu wire was only demonstrated for monomeric species[71] or poly(styrene) and poly(acrylate)star polymers,[72] we set up a series of reactions to optimize reaction conditions for the poly(amide)-based dendrons (Table 1). Because of the simplicity of the synthesis, **4** was used as the model for these studies, and variations on the reaction conditions include the use of sodium ascorbate as the reducing agent and the use of "click" additives such as SBP and pentamethyldiethylenetriamine (PMDETA) (Scheme 3 and Table 1).

When solely Cu wire was used (entry 1, Table 1), no CuAAC reaction was observed, and the solution presented a blue/greenish coloration, suggesting the presence of Cu(II) in solution (Cu(II) is not active in CuAAC). The reaction was then carried out in the presence of SA (entry 2, Table 1) to ensure the presence of Cu(I) in solution; however, despite the brown coloration of the solution, the CuAAC reaction did not proceed. To stabilize the Cu(I) species in solution, PMDETA was used (entries 3 and 4, Table 1). While this ligand has been shown to be efficient when used in combination with Cu wire,[72] it did not work for the poly(amide)-based dendrons. From all of the investigated CuAAC reaction conditions, only the use of SBP as ligand and SA as the reducing agent (entry 6, Table 1) resulted in the complete conversion between **4** and **14**. In this case, dendron tris-ETG **16** was isolated easily from the copper catalyst by removing the pieces of copper wire from the solution, evaporation of the $^t$BuOH/$H_2O$ solvent mixture, followed by dissolving the residue in methylene chloride, filtering it through Celite, and concentrating it under vacuum to yield a colorless waxy product in 92% yield. The relative high purity found for dendron tris-ETG **16** (for elemental analysis results, see the Experimental Section) encouraged us to apply the same conditions to the analogue dendron with nine branches.

We then applied the same reaction conditions (Cu wire/SA/SBP) to the CuAAC reaction between **8** and **14** (method C). However, the separation of **15** from the copper catalyst was as difficult as described for methods A and B. Despite the use of extensive washing, precipitation, and chromatography, the final dendron **15** synthesized by method C also presented a green coloration, suggesting contamination with copper. CHN elemental analysis of **15** synthesized by method C confirmed the nonpurity of the final product (Anal. Calcd for $C_{157}H_{276}N_{40}O_{50}$: C, 53.51; H, 7.89; N, 15.90. Found: C, 49.50; H, 7.16; N, 18.71). Contamination of **15** with copper was confirmed by ICP-MS analysis that showed a copper impurity of 0.004%.

For all synthetic CuAAC reactions studied, method A ($CuSO_4$/SA), method B (CuI/SA/SBP), and method C (Cu wire/SA/SBP), complete removal of the copper catalyst from **15** was not possible. While the use of SBP reduced the amount of copper contamination (0.007% Cu on method B and 0.004% Cu on method C) in comparison to method A (0.50% Cu), in all cases, the amount of copper present in dendron **15** is still significant, and the CHN elemental analysis suggests the presence of other unknown impurities. We suggest that the fact that **15** presents several groups capable of binding copper ions including amides, triazole, and PEG, together with the encapsulation ability of the dendritic structure, precludes the separation of **15** from copper residues. Indeed, it was shown that poly(amido amine) (PAMAM) dendrimers[74,75] and triazole-based dendrimers[36] bind different metal ions including $Cu^I$ and $Cu^{II}$ and that the dendritic structure plays in important role in the stability of the copper complexes formed inside the dendrimers. Because the final dendron nona-ETG **15** synthesized by CuAAC is a combination of poly(amide)-based and triazole-based dendritic structure, the inclusion of copper in its structure is not astonishing.

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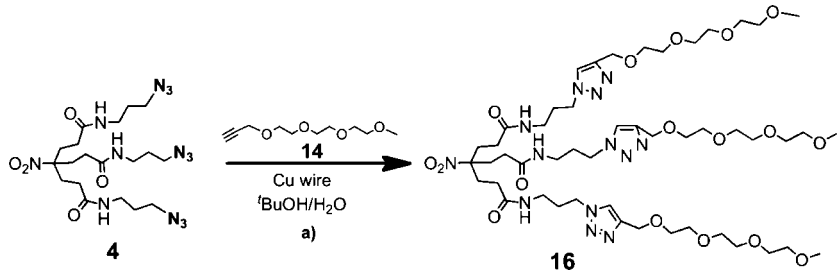
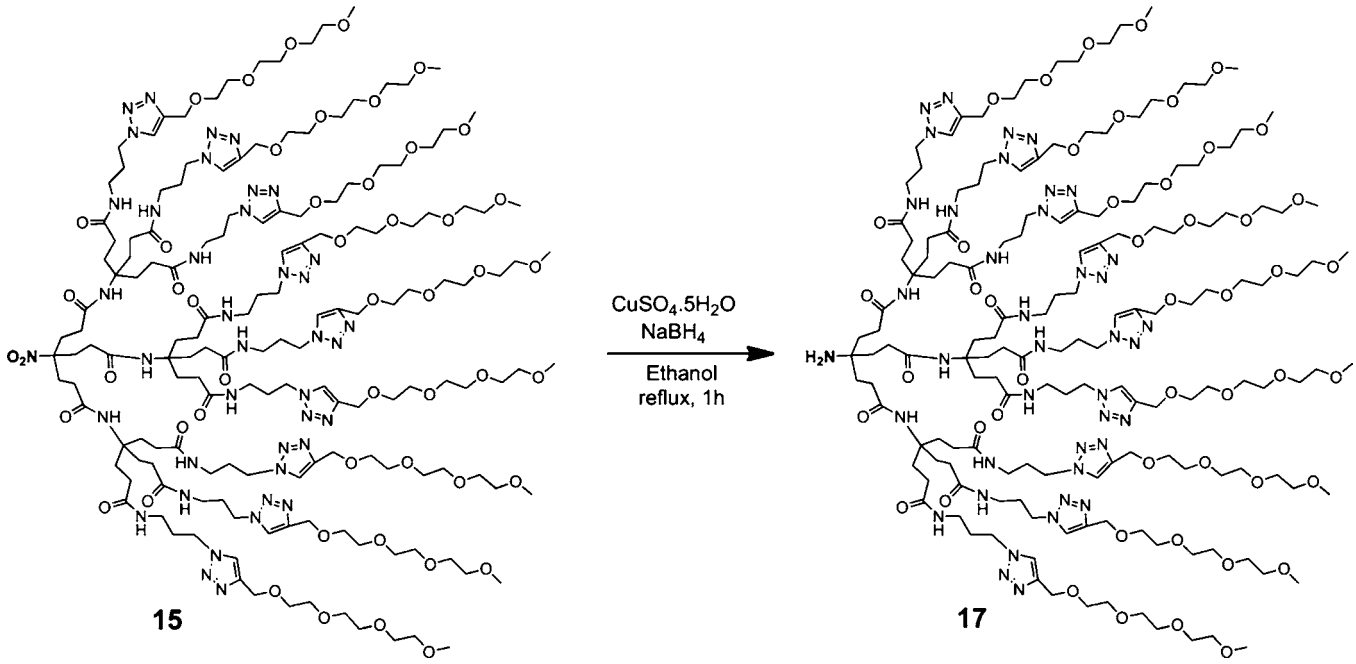
***Scheme 3.*** CuAAC Reaction between Dendron Tris-azide **4** and Alkyne Derivative **14** Using Copper Wire as the Cu(I) Source[a]




[a] The reaction conditions are described in Table 1.

***Scheme 4.*** Reduction of the Nitro Group at the Focal Point of Dendron **15**




As a final attempt to remove the copper residues from **15**, we carried out the reduction of the nitro group at the focal point to an amine, using a combination of $CuSO_4/NaBH_4$ in ethanol.[76] The reduction was carried out using **15** obtained from the three different methods described above. In all cases, during reflux the green colored solution became clear, and a black precipitate formed (presumably $Cu^0$). After removal of the black precipitate by filtration, dendron **17** was obtained as a light yellow waxy product (Scheme 4). The $^1H$ NMR spectrum of **17** shows all of the expected peaks including the triazole proton at 8.04 ppm and both C*H*$_2$-triazoles at 4.65 and 4.46 ppm. Formation of **17** was further confirmed by MALDI-TOF, showing its molecular ion peak $(M + Na)^+$ at 3517.1 *m*/*z* (calcd for $C_{157}H_{278}N_{40}O_{48}Na$: 3516.6 *m*/*z*). Despite the separation of a significant amount of copper by its precipitation as $Cu^0$, CHN elemental analysis of **17** still suggests the presence of a significant amount of copper and/or inorganic impurities (Anal. Calcd for $C_{157}H_{278}N_{40}O_{48}$: C, 54.97; H, 8.02. Found: C, 51.23; H, 7.21; the presence of Cu was confirmed by ICP-MS showing a copper content of 0.001%). Moreover, the harsh reactions conditions for the reduction step ($NaBH_4$/reflux) cannot be applied, generally limiting the potential of this strategy.

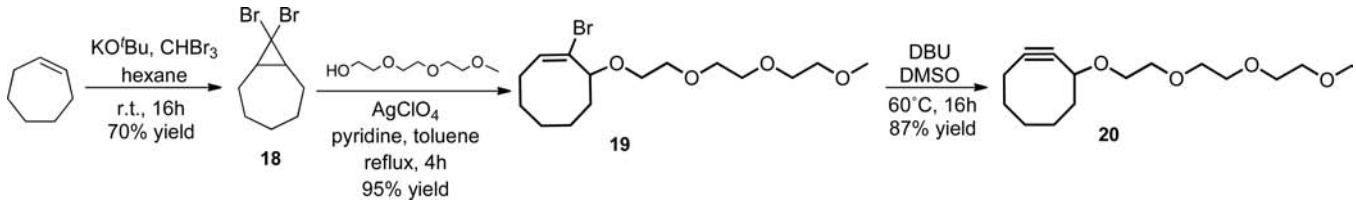
These results clearly raise a problem with Cu-catalyzed 1,3-dipolar cycloaddition when applied to macromolecular structures, in particular amide and PEG-based materials, which are highly desirable for biological applications. While CuAAC was shown to be a very useful tool in materials chemistry,[27,29] the significant copper contamination demonstrated in this contribution severely limits the use of CuAAC as the synthetic strategy of choice for the functionalization of poly(amide)-based materials. Incomplete copper removal prevents the use of these systems in biological applications due to the toxicity of copper, and this represents a step back for poly(amido)-based materials such as PAMAM.

Besides the CuAAC reaction conditions used here, procedures that include other "click" additives such as TBTA (tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine) or benzimidazole ligands could have been attempted; however, these ligands were shown to be efficient in accelerating CuAAC kinetics and in copper sequestering when used in monomeric species.[66−69] The fact that the copper wire/SA/SBP procedure afforded pure dendron tris-ETG but yielded copper contaminated dendron nona-ETG shows that the efficiency of "click" additives on monomeric species does not guarantee their success on dendritic molecules. Thus, our efforts were then focused on using a copper-free synthetic strategy: SPAAC.

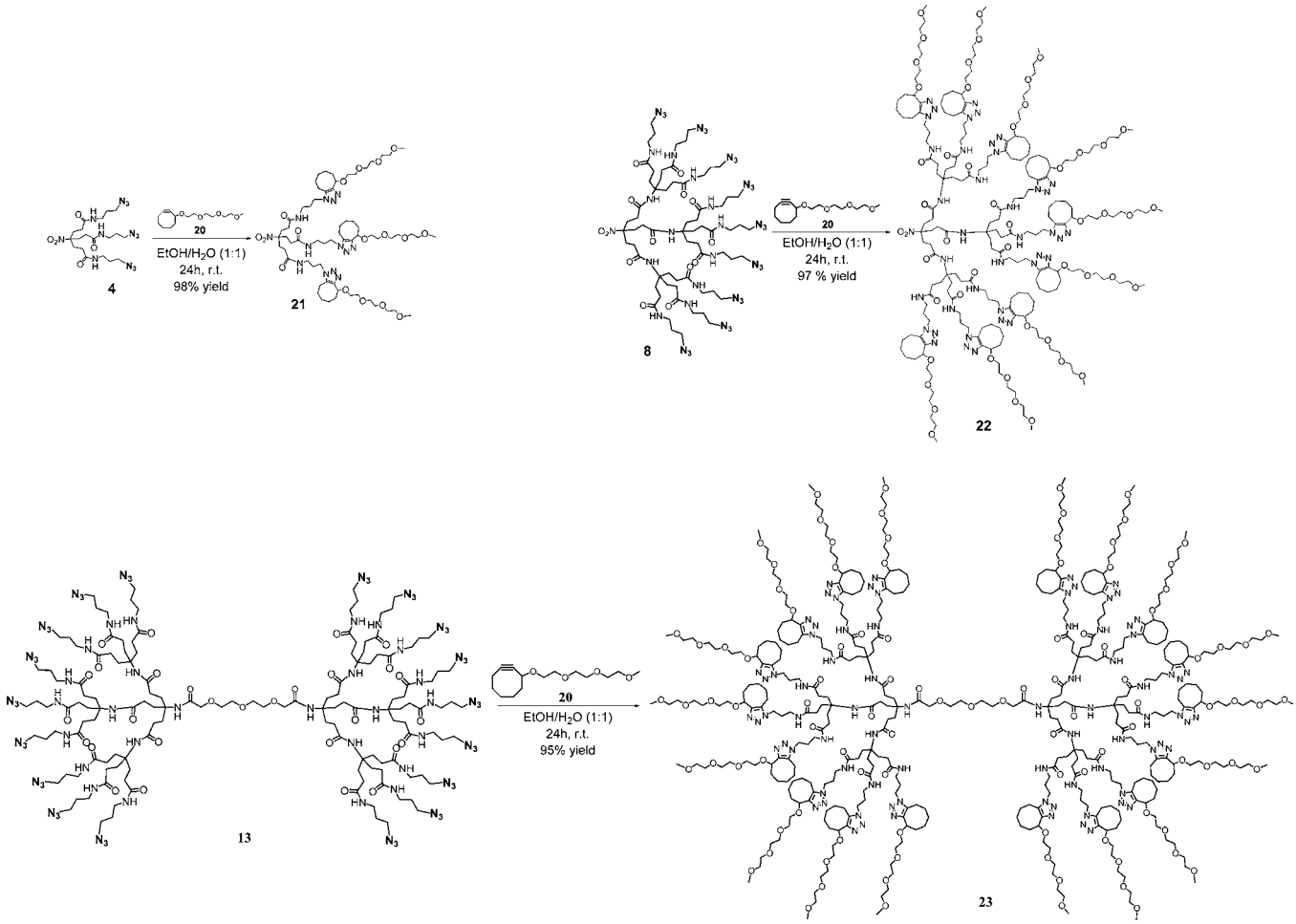
**Functionalization of Poly(amide)-Based Dendrons and Dendrimers with PEG Chains Using SPAAC.** As an alternative to CuAAC, we investigated the copper-free SPAAC, recently popularized by the Bertozzi group,[48] to functionalize our

(76) Sung-eun Yoo, S.-h. L. *Synlett* **1990**, *41*, 9–420.

***Scheme 5.*** Synthesis of Cyclooctyne-ETG **20**




***Scheme 6.*** SPAAC Reactions between Cyclooctyne-ETG **20** and Dendron Tris-azide **4**, Dendron Nona-azide **8**, and Dendrimer Eighteen-azide **13**[a]




[a] For clarity, only one of the triazole regioisomers is represented (for representation of both regioisomers, see Figure 1).

poly(amide)-based dendrons and dendrimers. Crucial to the use of SPAAC as a synthetic strategy is the preparation of functionalized cyclooctynes such as a PEG containing version that can be synthesized in three steps from commercially available cycloheptene.[50]

Bicyclic **18** (8,8-dibromobicyclo[5.1.0]octane) was synthesized in close analogy to the procedure described by Skattebol and Solomon for the synthesis of 9,9-dibromo[6.1.0]nonane.[53,77] Silver perchlorate was then used to carry out the electrocyclic ring-opening of **18** to the *trans*-allylic cation, which was captured with triethylene glycol monomethyl ether affording bromo-*trans*-cyclooctene **19**. The $^1$H NMR spectrum of **19** clearly shows a signal at 6.06 ppm characteristic of the vinyl proton. Compound **19** was also characterized by mass spectrometry showing its molecular ion peak $(M + Na)^+$ at 373.2 *m*/*z* (calcd for $C_{15}H_{27}BrO_4Na$: 373.1) with an isotopic distribution typical for bromine containing compounds. Cyclooctyne-ETG **20** was obtained in DMSO at 60 °C using DBU as the base (Scheme 5). Product **20** was characterized by mass spectrometry showing its molecular ion peak $(M + Na)^+$ at 293.3 *m*/*z* (calcd for $C_{15}H_{26}O_4Na$: 293.2) and by $^1$H and $^{13}$C NMR spectroscopies showing the characteristic C≡C signals at 100.8 and 93.9 ppm (for NMR and mass spectra of **19** and **20**, see the Supporting Information).

We initially investigated the SPAAC reaction between **20** and **4**, **8**, and **13** (Scheme 6). All three reactions were carried out in an EtOH/$H_2O$ (1:1) mixture using 1.5 equiv of **20** per azide group. At the beginning of each reaction, the solution was cloudy, probably due to the low solubility of the starting materials in the solvent mixture. However, after 30 min, the solutions became clear, and the $^1$H NMR spectra of the three reactions showed significant amounts of triazole products,

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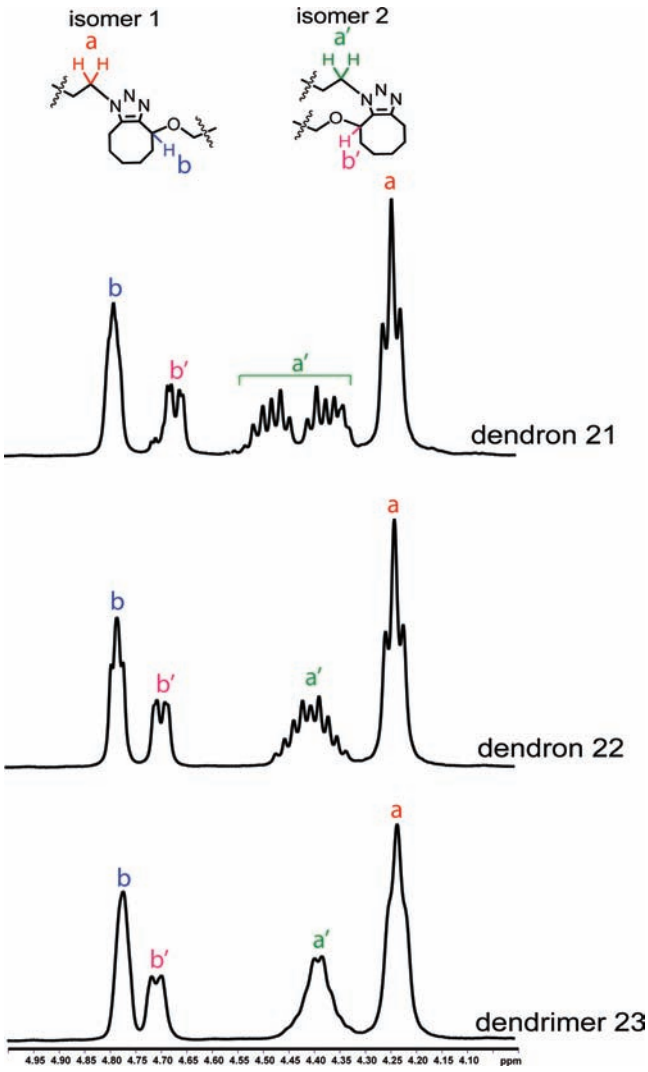


***Figure 1.*** Partial $^1$H NMR spectra of the SPAAC products showing signals of both regioisomers.

suggesting that the inclusion of some ETG chains into the dendrons and dendrimer increases their solubility in the aqueous solvent mixture. In all three cases, the solvents were removed under vacuum after 24 h, and the products were precipitated from $CH_2Cl_2$/hexane (Scheme 6). The $^1$H NMR spectra of the final products suggest completion of the SPAAC reaction after 24 h by complete disappearance of the C$H_2$N$_3$ signals at 3.36 ppm. The consumption of the azide termini was also confirmed by IR spectroscopy via the disappearance of the $N_3$ band at 2098 cm$^{-1}$. These results demonstrate the complete conversion of the azide groups to the triazole moieties.

The $^1$H NMR spectra of the SPAAC products present four new signals at 4.2−4.8 ppm. Assignment of the new signals was elucidated by COSY and HSQC NMR spectroscopies (for COSY and HSQC NMR spectra of dendrons **21** and **22** and dendrimer **23**, see the Supporting Information). By comparison with the azide derivatives, the signal corresponding to NHC$H_2$CH$_2$CH$_2$-triazole protons can be identified easily at 3.22 pm. Analyzing the 2D COSY NMR spectrum, we observed the coupling between the NHC$H_2$CH$_2$CH$_2$-triazole protons with the NHCH$_2$C$H_2$CH$_2$-triazole protons that appear at 2.03 ppm. The coupling between NHCH$_2$C$H_2$CH$_2$-triazole and the C$H_2$-triazole protons shows that the C$H_2$-triazole protons appear at 4.39 and 4.23 ppm. The assignment of the two different signals to the C$H_2$-triazole protons suggests the presence of the two expected regioisomers. Analyzing the HSQC NMR spectra of dendrimer **23** allows for the assignment of the signals at 4.77 and 4.70 ppm to the C*H* protons of cycloctyne adjacent to triazole moiety, again suggesting the presence of both regioisomers.

Once the proton signals for both triazole regioisomers were assigned, their ratio was calculated on the basis of integrations. The C$H_2$-triazole signals (labeled a and a′ in Figure 1) appear as a triplet at 4.24 ppm for isomer 1 and as a multiplet at 4.30−4.55 ppm for isomer 2. The signal of the allyl protons adjacent to the ETG chain (labeled b and b′ in Figure 1) appears as a triplet at 4.79 ppm for isomer 1 and as a multiplet at 4.63−4.70 ppm for isomer 2. Previous studies about SPAAC kinetics and isomerization ratios in monomeric units showed that both isomers are formed in approximately 1:1 ratios.[50] In our case, the ratio of isomer 1/isomer 2 for dendron tris-ETG **21** is 1:1.2 (55% isomer 1 and 45% isomer 2), which is comparable to the results obtained for the monomeric species. Dendron **22** and dendrimer **23** present ratios of approximately 1:1.7 (63% isomer 1 and 37% isomer 2), suggesting that the steric hindrance favors the formation of isomer 1 (Figure 1). To study the influence of the temperature on regioisomers formation, the SPAAC reaction was also carried out at 45 and 80 °C. At these temperatures, nearly quantitative yields were also obtained, but no difference was observed in the final regioisomer ratio.

The presence of both isomers on the SPAAC products was also detected by $^{13}$C NMR spectroscopy where four different signals for the two quaternary carbons of the triazole ring (145.2, 144.5, 134.1, and 133.4 ppm) were obtained (see the Supporting Information).

Mass spectrometry was used to further verify the products of the SPAAC reactions. Dendron tris-ETG **21** was characterized by ESI mass spectrometry, and its molecular ion peak $(M + Na)^+$ was observed at 1356.9 (calcd for $C_{64}H_{111}N_{13}O_{17}Na$: 1356.8). Dendron nona-ETG **22** and dendrimer eighteen-ETG **23** presented their molecular ion peaks by MALDI-TOF mass spectrometry at $(M + H)^+$ at 4135.8 (calcd for $C_{202}H_{349}N_{40}O_{50}$: 4134.6) and 8396.1 (calcd for $C_{412}H_{711}N_{80}O_{101}$: 8396.3), respectively (Figure 2). Because of the higher molecular weight of dendrimer **23**, higher laser power was needed to obtain the molecular ion peak, and thus significant fragmentation was observed. The difference between the fragmentation peaks is 335, which corresponds to the loss of a triazole-ETG fragment (see the Supporting Information for details on fragmentation).

In stark contrast to the CuAAC products, all SPAAC products were obtained analytically pure when submitted to elemental analysis (For dendron **21**, Anal. Calcd for $C_{64}H_{111}N_{13}O_{17}$: C, 57.59; H, 8.38. Found: C, 57.68; H, 8.42. For dendron **22**, Anal. Calcd for $C_{202}H_{349}N_{40}O_{50}$: C, 58.63; H, 8.48. Found: C, 58.77; H, 8.51. For dendrimer **23**:, Anal. Calcd for $C_{412}H_{710}N_{81}O_{101}$: C, 58.91; H, 8.52. Found: C, 59.11; H, 8.59). These results clearly demonstrate the superiority of SPAAC in the functionalization of amide-based materials including dendrimers over CuAAC, allowing the use of such materials in biological applications. The SPAAC reaction showed to be efficient on dendrimers up to 18 azide

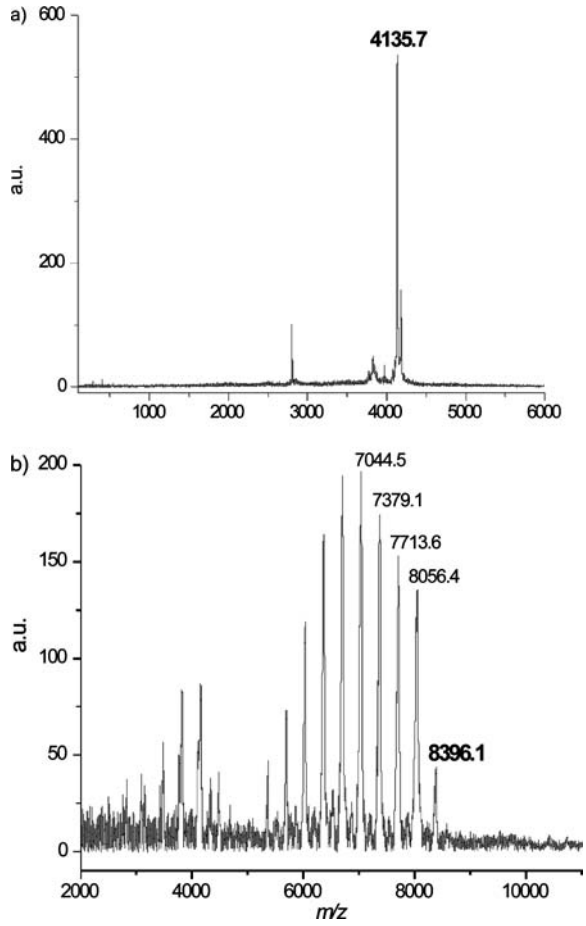


***Figure 2.*** MALDI-TOF mass spectra of (a) dendron **22** and (b) dendrimer **23** (due to the higher molecular weight of **23**, higher laser power was needed to obtain the molecular ion peak, which is probably the cause of the significant fragmentation peaks).

termini, which makes this reaction a new useful synthetic tool for dendrimer functionalization.

## Conclusion

This contribution describes the advantage of strain-promoted alkyne azide 1,3-dipolar cycloaddition in comparison to the copper-catalyzed version for the functionalization of poly(amide)-based dendrons and dendrimers, promising materials for a wide variety of biological applications. In particular, mild reaction conditions, complete conversions, and the lack of residual copper make SPAAC the functionalization strategy of choice. The SPAAC products were characterized by $^{1}H$ and $^{13}C$ NMR, 2D HSQC, and COSY NMR spectroscopies, mass spectrometry, and elemental analysis. All characterization methods demonstrate clean reactions and complete conversions. This is the first report on the use of such a reaction on dendrimers functionalization, and the results obtained here demonstrate that this reaction can be an important tool in dendrimer chemistry due to the mild and metal-free reaction conditions, no side products, tolerance toward functional groups, and high yields. Application of this synthetic tool toward multifunctional dendrimers to build well-defined drug delivery carriers for theranostics is currently under investigation in our group.

## Experimental Section

**General.** Dendrons nitrotriester **1** and aminotriester **5** were purchased from Frontier Scientific. Dendron triacid **2** was obtained by deprotection of the acid groups on commercial dendron nitrotriester **1** as previously described.[61,62,78] Dendrons **6**, **7**, and **9** were prepared following literature procedures (for detailed experimental procedures and characterization data of all compounds, see the Supporting Information).[61,62,78] 3-Aminopropyl azide **3** was synthesized according to literature procedures.[79]

**Synthesis of Dendrimer 23.** Dendrimer eighteen-azide **13** (0.013 g, 0.0037 mmol) and cyclooctyne **20** (0.027 g, 0.100 mmol) were dissolved in 1 mL of ethanol. Water was added (1 mL), and the solution became cloudy. The solution was stirred at room temperature for 24 h, resulting in a colorless solution. The solvent was removed, and the product was washed twice with hexanes and precipitated from $CH_2Cl_2$/hexanes to remove the cyclooctyne that was added in excess. Dendrimer **23** was obtained as a colorless waxy product in 95% yield (0.030 g). $^{1}H$ NMR ($CDCl_3$, 400 MHz, $\delta_{ppm}$ vs TMS): 7.76 (s, CON*H*, 26H), 4.77 (s, *C*═C*H*−O, isomer 1), 4.70 (m, *C*═C*H*−O, isomer 2), 4.39 (m, C$H_2$-triazole, isomer 2), 4.23 (t, $J$ = 6.84 Hz, C$H_2$-triazole, isomer 1), 3.89 (s, OC$H_2$CONH, 4H), 3.65−3.45 (m, OC$H_2$C$H_2$O, 224H), 3.34 and 3.32 (s, OC$H_3$, 54H), 3.22 (m, CONHC$H_2$, 36H), 3.06−2.62 (m, C$H_2$C═CHC$H_2$ of cyclooctene ring, 72H), 2.25, 2.16, and 1.96 (m, C$H_2$C$H_2$NHCO, 96H), 2.03 (m, CONHC$H_2$, 36H), 1.77−0.97 (m, C$H_2$ of cyclooctene ring, 108H). $^{13}C$ NMR ($CDCl_3$, 100 MHz, $\delta_{ppm}$ vs TMS): 174.2−173.4 (*C*═O), 145.2 and 144.5 ($CH_2NC_q$ of triazole ring, both isomers), 134.1 and 133.4 ($CH_2N_3C_q$ of triazole ring, both isomers), 74.7 and 72.0 (*C*H−O of cyclooctene ring), 70.4−70.6 and 68.0 (OC$H_2$C$H_2$O and OC$H_2$NHCO), 59.2 (OC$H_3$), 46.7 and 45.5 (C$H_2$-triazole, both isomers), 37.0 ($CH_2CH_2CH_2$-triazole), 35.6 ($CH_2CH_2CH_2$-triazole), 31.7−20.2 (C$H_2$ of cyclooctene ring and C$H_2$C$H_2$CONH). MALDI-TOF $(M + H)^+$ $m/z$ calcd for $C_{412}H_{710}N_{80}O_{101}$: 8396.1, found 8395.3. Anal. Calcd for $C_{412}H_{710}N_{81}O_{101}$: C, 58.91; H, 8.52. Found: C, 59.11; H, 8.59.

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**Supporting Information Available:** Detailed synthetic procedures, and NMR and mass spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JA910581D

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