

Construction of a Well-Defined Multifunctional Dendrimer for Theranostics

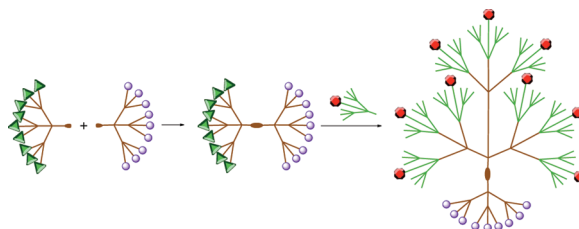
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ABSTRACT



A dendrimer-based building block for theranostics was designed. The multifunctional dendrimer is polyamide-based and contains nine azide termini, nine amine termini, and fifty-four terminal acid groups. Orthogonal functionalization of the multifunctional dendrimer with a near-infrared (NIR) cyanine dye afforded the final dendrimer that shows fluorescence in the NIR region and no toxicity toward T98G human cells. The synthetic strategy described here might be promising for fabricating the next generation of materials for theranostics.

Dendrimers are perfectly hyperbranched macromolecules that possess a high number of active termini that define their properties and functions.^{1–5} They have found applications in material science as sensors,^{6,7} nanoreactors,^{8,9} and green catalyst supports.¹⁰ The most promising applications of dendrimers are in biomaterials where they have

emerged as competitive candidates for drug delivery applications.^{5,11–14}

Traditionally, dendrimers are synthesized from AB_x monomers, resulting in symmetrical structures with B terminal groups.¹⁵ However, such symmetrical dendritic structures cannot be employed easily in complex applications such as theranostics and drug delivery. For example, materials for theranostics need higher structural complexity because multiple functionalities for water solubility, targeting, imaging, and drug delivery are required.¹⁴ Clearly, the multifunctionalization of dendrimers is crucial for the construction of dendrimer-based drug delivery systems.

Multifunctionalization of poly(amidoamine) (PAMAM) dendrimers, the leading dendrimer scaffold in biomaterials, is mainly achieved through a random statistical

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(1) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A. *Angew. Chem., Int. Ed.* **1990**, *29*, 138.

(2) Fréchet, J. M. J. *Science* **1994**, *263*, 1710.

(3) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendrimers and Dendrons: Concepts, Synthesis, Applications*; Wiley-VCH: Weinheim, 2001.

(4) Astruc, D. *Dendrimers and nanosciences - Foreword*; Elsevier: Paris, 2003.

(5) Astruc, D.; Boisselier, E.; Ornelas, C. *Chem. Rev.* **2010**, *110*, 1857.

(6) Ornelas, C.; Aranzaes, J. R.; Cloutet, E.; Alves, S.; Astruc, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 872.

(7) Astruc, D.; Ornelas, C.; Ruiz, J. *Acc. Chem. Res.* **2008**, *41*, 841.

(8) Crooks, R. M.; Zhao, M. Q.; Sun, L.; Chechik, V.; Yeung, L. K. *Acc. Chem. Res.* **2001**, *34*, 181.

(9) Ornelas, C.; Aranzaes, J. R.; Salmon, L.; Astruc, D. *Chem.—Eur. J.* **2008**, *14*, 50.

(10) Mery, D.; Astruc, D. *Coord. Chem. Rev.* **2006**, *250*, 1965.

(11) Lee, C. C.; MacKay, J. A.; Fréchet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, *23*, 1517.

(12) Tomalia, D. A.; Reyna, L. A.; Svenson, S. *Biochem. Soc. Trans.* **2007**, *35*, 61.

(13) Wolinsky, J. B.; Grinstaff, M. W. *Adv. Drug Delivery Rev.* **2008**, *60*, 1037.

(14) Tekade, R. K.; Kumar, P. V.; Jain, N. K. *Chem. Rev.* **2009**, *109*, 49.

(15) Newkome, G. R.; Shreiner, C. D. *Polymer* **2008**, *49*, 1.

approach,^{14,16} i.e. by successive partial functionalization of the amine termini, resulting in a stochastic distribution of products without any degree of control over individual functional group placement.¹⁷ Indeed, Baker and co-workers have recently showed that the random functionalization of dendrimers leads to a complex mixture of products that is far from following a Gaussian distribution and that in most cases only a small percentage of the dendrimers have the desired multifunctionality.¹⁷ While highly desirable, synthetic strategies to selectively multifunctionalize dendrimers are limited.^{15,18–26}

Our group has reported the synthesis of trifunctional poly(amide)-based dendrimers and has demonstrated their orthogonal functionalization by using either the copper-catalyzed azide alkyne 1,3-dipolar cycloaddition (CuAAC) or the strain promoted azide alkyne cycloaddition (SPAAC) combined with Schiff base coupling.^{27–30} In this contribution, we describe the basic building blocks for a dendrimer-based multifunctionalization strategy that has the potential to be utilized in theranostics and other biomaterials applications. We report a synthetic strategy toward multifunctional dendrimers that consists of the combination of two different dendrons into a Janus-like^{18,31–35} dendrimer, followed by the reaction with an AB₆C₁ dendron resulting in a well-defined hyperbranched multifunctional dendrimer.

To demonstrate our strategy, we report the synthesis of a dendrimer, bearing fifty-four acids, nine amines, and nine azide groups, that has the potential to be multifunctionalized in three steps (Scheme 1). As a proof-of-principle, we demonstrate the orthogonal monofunctionalization by selectively reacting the nine amine termini with a near-infrared (NIR) fluorescent dye.

Among the most promising NIR dyes^{36,37} are cyanine-based dyes.^{38–44} Recently, Gao and co-workers have shown that when the chlorine atom of the cyanine dyes is replaced by an amine group, the resulting amino-cyanine dyes have high quantum yields and large Stokes shifts (> 140 nm).⁴⁵ Our group has demonstrated that the S_{RN}1 reaction between the chloride-containing cyanine dye and functionalized amines is fully orthogonal to azides and acid groups.⁴⁶ To increase water solubility, we select here a cyanine dye that contains two sulfonate groups at the end of the propyl chains on the nitrogen of the indolenine ring. Furthermore, the negative charges on the sulfonate groups should enhance the biocompatibility of the dye–dendrimer conjugate since it has been demonstrated that negatively charged dendrimers are less toxic than their positively charged counterparts.^{13,14}

The dendrons used in our strategy are poly(amide)-based to ensure biocompatibility and follow the 1→(2+1) connectivity pioneered by Newkome.^{20,26} All peptide coupling reactions described here were achieved successfully using HATU as the coupling agent, and all dendrimers were purified easily by dialysis using a Spectra-Por MWCO (molecular weight cut off) 2000 dialysis membrane.

The basic synthesis toward our target dendrimer **7** is shown in Scheme 1. The dendrimer design is based on dendron **1** containing nine protected acid groups and one acid group at the focal point and dendron **2** containing nine azide groups and one amine at the focal point. Both dendrons were synthesized according to our previous report²⁹ (see Supporting Information (SI) for experimental details). The coupling reaction between **1** and **2** afforded dendrimer **3** in 93% yield after purification by dialysis against methanol. The quantitative coupling was proven by ¹H NMR spectroscopy with the shifting of the CH₂-COOH protons on **1** from 2.63 to 2.55 ppm as well as the triplet corresponding to the CH₂CH₂NH₂ protons of **2** from 3.77 to 3.60 ppm upon formation of **3** (the CH₂NH₂ protons cannot be used to monitor the reaction because they overlap with the CH₂N₃ protons). Dendrimer **3** presents its molecular ion peak (M+H)⁺ at 3340.5 m/z (calcd for C₁₅₃H₂₆₄N₄₅O₃₈: 3340.0 m/z) in the MALDI-TOF mass spectrum corroborating the expected structure. The acid termini on **3** were deprotected at room temperature using

(16) Majoros, I. J.; Williams, C. R.; Baker, J. R. *Curr. Top. Med. Chem.* **2008**, *8*, 1165.

(17) Mullen, D. G.; Fang, M.; Desai, A.; Baker, J. R.; Orr, B. G.; Banaszak Holl, M. M. *ACS Nano* **2010**, *4*, 657.

(18) Maraval, V.; Laurent, R.; Merino, S.; Caminade, A.-M.; Majoral, J.-P. *Eur. J. Org. Chem.* **2000**, 3555.

(19) Zhang, W.; Nowlan, D. T.; Thomson, L. M.; Lackowski, W. M.; Simanek, E. E. *J. Am. Chem. Soc.* **2001**, *123*, 8914.

(20) Newkome, G. R.; Kim, H. J.; Moorefield, C. N.; Maddi, H.; Yoo, K. S. *Macromolecules* **2003**, *36*, 4345.

(21) Sivanandan, K.; Sandanaraj, B. S.; Thayumanavan, S. *J. Org. Chem.* **2004**, *69*, 2937.

(22) Lee, J. W.; Kim, B. K.; Kim, J. H.; Shin, W. S.; Jin, S. H. *J. Org. Chem.* **2006**, *71*, 4988.

(23) Goodwin, A. P.; Lam, S. S.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2007**, *129*, 6994.

(24) Franc, G.; Kakkar, A. *Chem. Commun.* **2008**, 5267.

(25) Antoni, P.; Hed, Y.; Nordberg, A.; Nystrom, D.; von Holst, H.; Hult, A.; Malkoch, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 2126.

(26) Newkome, G. R.; Shreiner, C. *Chem. Rev.* **2010**, *110*, 6338.

(27) Goyal, P.; Yoon, K.; Weck, M. *Chem.—Eur. J.* **2007**, *13*, 8801.

(28) Yoon, K.; Goyal, P.; Weck, M. *Org. Lett.* **2007**, *9*, 2051.

(29) Ornelas, C.; Weck, M. *Chem. Commun.* **2009**, 5710.

(30) Ornelas, C.; Broichhagen, J.; Weck, M. *J. Am. Chem. Soc.* **2010**, *132*, 3923.

(31) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1993**, *115*, 11496.

(32) Saez, I. M.; Goodby, J. W. *Chem. Commun.* **2003**, 1726.

(33) Ropponen, J.; Nummelin, S.; Rissanen, K. *Org. Lett.* **2004**, *6*, 2495.

(34) Fuchs, S.; Pla-Quintana, A.; Mazères, S.; Caminade, A.-M.; Majoral, J.-P. *Org. Lett.* **2008**, *10*, 4751.

(35) Wu, P.; Malkoch, M.; Hunt, J. N.; Vestberg, R.; Kaltgrad, E.; Finn, M. G.; Fokin, V. V.; Sharpless, K. B.; Hawker, C. J. *Chem. Commun.* **2005**, 5775.

(36) Bouteiller, C.; Clave, G.; Bernardin, A.; Chipon, B.; Massonneau, M.; Renard, P.-Y.; Romieu, A. *Bioconjugate Chem.* **2007**, *18*, 1303.

(37) Sameiro, M.; Goncalves, T. *Chem. Rev.* **2009**, *109*, 190.

(38) Mishra, A.; Behera, R. K.; Behera, P. K.; Mishra, B. K.; Behera, G. B. *Chem. Rev.* **2000**, *100*, 1973.

(39) Narayanan, N.; Patonay, G. *J. Org. Chem.* **2002**, *60*, 2391.

(40) Ye, Y. P.; Bloch, S.; Kao, J.; Achilefu, S. *Bioconjugate Chem.* **2005**, *16*, 51.

(41) Almutairi, A.; Guillaudeu, S. J.; Berezin, M. Y.; Achilefu, S.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2008**, *130*, 444.

(42) Almutairi, A.; Akers, W. J.; Berezin, M. Y.; Achilefu, S.; Fréchet, J. M. J. *Mol. Pharmaceutics* **2008**, *5*, 1103.

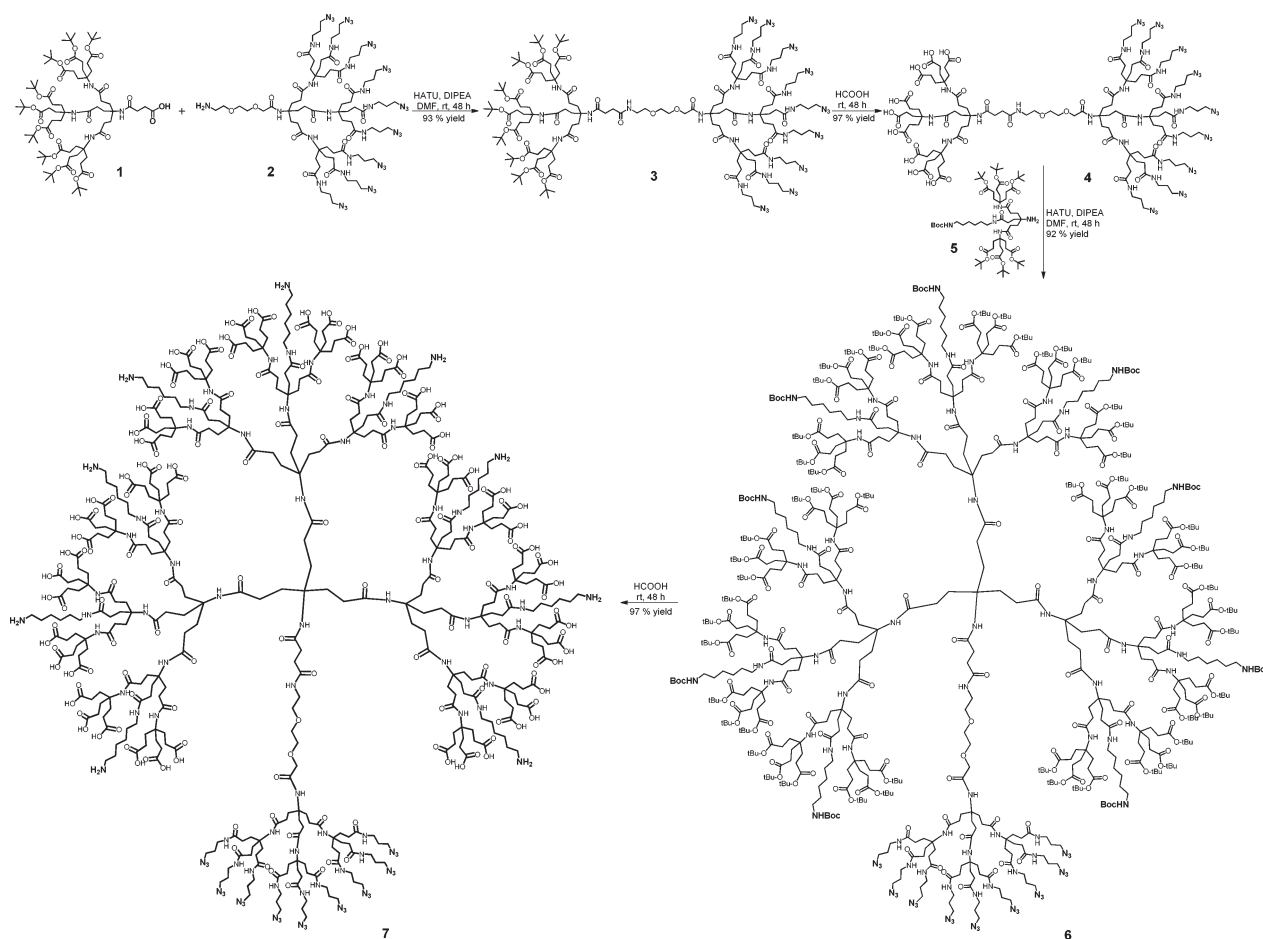
(43) Talanov, V. S.; Regino, C. A. S.; Kobayashi, H.; Bernardo, M.; Choyke, P. L.; Brechbiel, M. W. *Nano Lett.* **2006**, *6*, 1459.

(44) Saad, M.; Garbuzenko, O. B.; Ber, E.; Chandna, P.; Khandare, J. J.; Pozharov, V. P.; Minko, T. *J. Controlled Release* **2008**, *130*, 107.

(45) Peng, X.; Song, F.; Lu, E.; Wang, Y.; Zhou, W.; Fan, J.; Gao, Y. *J. Am. Chem. Soc.* **2005**, *127*, 4170.

(46) Ornelas, C.; Lodscar, R.; Durandin, A.; Canary, J.; Pennell, R.; Liebes, L.; Weck, M. *Chem.—Eur. J.* **2011**.

Scheme 1. Synthesis of Multifunctional Dendrimer **7**: Combination of Two Different Dendrons into a “Janus”-Type Dendrimer Followed by the Reaction with an AB₆C₁ Dendron



formic acid, resulting in the “Janus”-type dendrimer **4** that possesses nine acid groups and nine azide groups. Complete deprotection was proven *via* ¹H NMR spectroscopy by the disappearance of the *t*Bu protons at 1.44 ppm and the appearance of COOH protons at 8.41 ppm. The structure of **4** was further confirmed by mass spectrometry (MALDI-TOF) showing its molecular ion peak (M+H)⁺ at 2835.5 *m/z* (calcd for C₁₁₇H₁₉₂N₄₅O₃₈: 2835.4 *m/z*).

Multifunctional AB₆C₁ dendron **5**, where A = NH₂; B = COO*t*Bu; C = NHBoc (for the synthesis of **5**, see SI), was then coupled to the nine acid groups on **4** yielding dendrimer **6** that contains fifty-four protected acids, nine Boc-protected amines, and nine azide groups. Again, **6** was purified easily by dialysis against methanol using a Spectra-Por MWCO 2000 dialysis membrane and isolated as a colorless waxy product in 92% yield. The ¹H NMR spectrum proves the structure of **6**, showing all the expected peaks that include the PEG linker between 4.0 and 3.59 ppm, CH₂N₃ at 3.37 ppm, and CH₂NHBoc at 3.04 ppm (for NMR spectra and data, see SI). The acid and the amine groups on **6** were simultaneously deprotected at room temperature

using formic acid. The quantitative deprotection reaction was proven by ¹H and ¹³C NMR spectroscopies *via* the disappearance of the *t*Bu protons at 1.44 ppm as well as the disappearance of the COO*t*Bu carbon signals at 174.6 ppm (CH₂COO*t*Bu, protected acids) and at 158.7 ppm (CH₂NHCOO*t*Bu, protected amines). The resulting dendrimer **7** is fully water-soluble and has on its periphery fifty-four acid groups, nine amine groups, and nine azide groups, suitable for further orthogonal functionalization.

Orthogonal functionalization of **7** was carried out by reacting the nine amine groups of **7** through an S_{RN}1 reaction with the chlorine-cyanine NIR-dye **8** (for synthesis of NIR-dye **8**, see SI) (Scheme 2).^{45–47}

The reaction was carried out in DMF at 80 °C for 48 h in which the initial deep green solution slowly turned to deep blue suggesting the substitution of the chloride atoms by the amine groups. The solution was then evaporated to dryness, and the product was purified by dialysis against methanol using a Spectra-Por MWCO 2000 dialysis membrane. Dendrimer **9** was obtained as a deep blue waxy product in 87% yield (Figure 1).

Dendrimer **9** was characterized by NMR spectroscopy, mass spectrometry, and UV–vis and fluorescence spectroscopies. The 2D COSY NMR spectra of **9**

(47) Flanagan, J. H.; Khan, S. H.; Menchen, S.; Soper, S. A.; Hammer, R. P. *Bioconjugate Chem.* **1997**, *8*, 751.

Scheme 2. Functionalization of Chlorine-Cyanine Dye **8** with Amine Derivatives

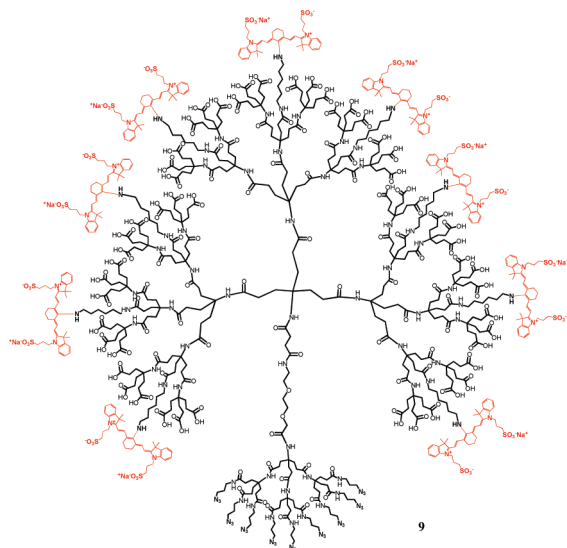
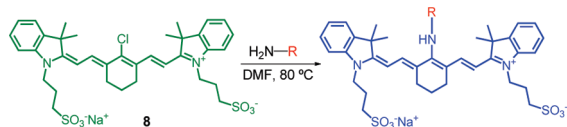


Figure 1. Dendrimer **9** containing nine NIR-dyes, nine azide termini, and fifty-four acid termini.

show the coupling between the CH_2NH -dye protons at 2.95 ppm and the cyanine protons at 8.04 ppm suggesting the proximity of both moieties. The CH_2N_3 signal at 3.35 ppm and the CH_2COOH signal at 2.29 ppm do not show any coupling with the protons from the NIR-dye demonstrating that **8** did not react with either the acid or the azide groups on **9**, confirming the orthogonality of the $\text{S}_{\text{RN}}1$ reaction and the presence of these functional groups as dendrimer termini. The MALDI-TOF mass spectra of **9** show its molecular ion peak M^{2+} at 8037.6 m/z (M^{2+} calculated for $\text{C}_{765}\text{H}_{1091}\text{N}_{108}\text{Na}_9\text{O}_{218}\text{S}_{18}$: 8032.6 m/z).

The optical properties of **9** were investigated by UV-vis spectroscopy in different solvents: phosphate buffer aqueous solution (PBS), dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethanol, and methanol (Figure 2). All UV-vis spectra are characterized by a broad band around 619–641 nm showing a large blue shift in comparison to that of the parent dye **8**, which is typical for the amino-cyanine dyes.^{45,46} The absorption spectra of **9** also present a narrow absorption band at around 770–793 nm, suggesting the presence of J-aggregates.^{38,48} The intensity of this band is much less significant in PBS than in other solvents, suggesting that the aggregation phenomena are lower in aqueous media. This is probably due to the

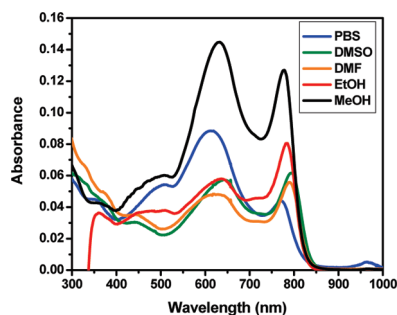


Figure 2. Absorption spectra of dendrimer **9** in five different solvents.

hydrophilicity of the dendrimer's periphery and is an interesting feature taking into account the potential for biological applications. Dendrimer **9** is fluorescent in the NIR-region, its emission spectra showing one emission band at 762 nm with comparable quantum yield (0.062) to the FDA approved dye, indocyanine green (0.078). The large Stokes shift (~ 140 nm), the emission on the NIR-region, and the low aggregation in aqueous media are optimal properties for *in vivo* optical imaging.

Since our main motivation is the development of a basic dendrimer building block that can be orthogonally multifunctionalized for applications in theranostics, it is essential to assess the cytotoxicity of **9**. The toxicity of **9** was evaluated for T98G human cells that were grown in the presence of different concentrations of **9**. The assay demonstrates that, even after an incubation time of 72 h, **9** is not significantly toxic for T98G human cells at concentrations up to 50 μM . (see SI p S-35).

In summary, we have designed and synthesized a new dendrimer-based building block for biomaterials applications. The well-defined dendrimer is water-soluble and can be multifunctionalized in a highly controllable and orthogonal fashion. The multifunctional dendrimer is polyamide-based and contains nine azide termini, nine amine termini, and fifty-four terminal acid groups. Orthogonal functionalization of the multifunctional dendrimer was achieved by the reaction of the amine termini with a NIR-cyanine dye. The final dye-containing dendrimer shows fluorescence in the NIR region with a large Stokes shift and relatively high quantum yields and shows no toxicity toward T98G human cells. The results obtained here are promising for the application of these materials in theranostics. Current efforts in our group are focused on the attachment of targeting moieties to the azide termini and cancer drugs to the acid groups on these dendrimers.

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Supporting Information Available. Detailed synthetic procedures, characterization data, ^1H NMR and mass spectra for all compounds; emission spectra and toxicity evaluation of dendrimer **9**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

(48) Jelly, E. E. *Nature* **1936**, 138, 1009.