VIP Supramolecular Chemistry

DOI: 10.1002/ange.200804987

Bifunctional Dendrimers: From Robust Synthesis and Accelerated One-Pot Postfunctionalization Strategy to Potential Applications**

*Per Antoni, Yvonne Hed, Axel Nordberg, Daniel Nyström, Hans von Holst, Anders Hult, and Michael Malkoch**

Dendritic polymers including hyperbranched materials, dendronized polymers, dendrigrafts, and dendrimers have emerged as a promising family of macromolecules that complement linear polymers. These structures are expected to play a crucial role in future cutting-edge applications as they display extraordinarily high functional group density per macromolecule. Dendrimers are the flagship for dendritic polymers and typically range from 1 to 10 nm in size. They are monodispersed and constructed by using repetitive steps of efficient synthetic protocols, either by the divergent[1,2] or convergent approach.[3] Traditional dendrimers are synthesized from AB_x monomers, and the resulting structures can be thought of as reactive scaffolds which comprise inactive interiors and active exteriors having multiple functional groups (Figure 1 A). However, as dendritic materials migrate into new research fields the demand on their structural complexity is increasing. For example, recently proposed delivery systems in the field of biotechnology include the construction of sophisticated dendritic delivery vehicles possessing two different functional groups (Figure 1 B).[4–6] The delivery systems are composed of a dendron wedge having A-type functionality, which allows the target to reach its destination, whereas the second dendron wedge having B-type functionality expresses multiple active drug compounds[4] or fluorescent dyes for quantitative measurements.[5] The potential for bifunctional dendrimers is great; however, the synthetic protocols utilized to obtain such structures are tedious and typically require a minimum of 16 sequential reaction steps to a obtain fourth generation dendrimer having a total of 32 (16+16) reactive groups. For these sophisticated dendrimers to become commercially viable, the number of reaction steps needs to be reduced while maintaining a high number of functional groups. Unfortunately, only a limited number of publications are available which report on accelerated synthetic methodologies.[7] An alternative approach was recently proposed for the construction of peripheral bifunctional dendrimers.[8] The strategy included the endcapping of 2,2-bis(methylol)propionic acid (bisMPA) dendrimers with a cyclic carbonate monomer which was then transformed to produce bifunctionality on the periphery. A fourth generation dendrimer having 48 functional groups (24+24) was obtained in eight steps (Figure 1 C).

To take advantage of the dendritic framework it is evident that the typically dormant dendritic interior needs to be activated by incorporating anchored functional groups which

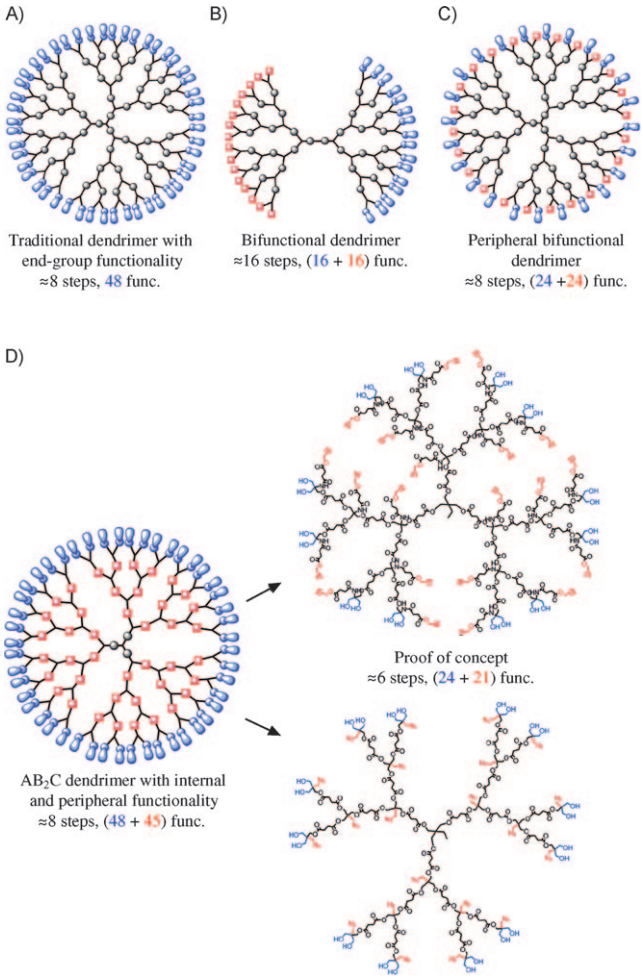


Figure 1. Dendrimer evolution. A comparison of different dendritic architectures and functionalities.

[*] Dr. P. Antoni, Y. Hed, Dr. D. Nyström, Prof. A. Hult, Prof. M. Malkoch
Royal Institute of Technology
School of Chemistry and Chemical Science
Division of Coating Technology
Teknikringen 56-58, 10044 Stockholm (Sweden)
Fax: (+46) 790-8283
E-mail: malkoch@kth.se
A. Nordberg, Prof. H. von Holst
Royal Institute of Technology, School of Technology and Health
Neuronic Engineering, Huddinge (Sweden)
and Karolinska Institute
Department of Clinical Neuroscience, Stockholm (Sweden)

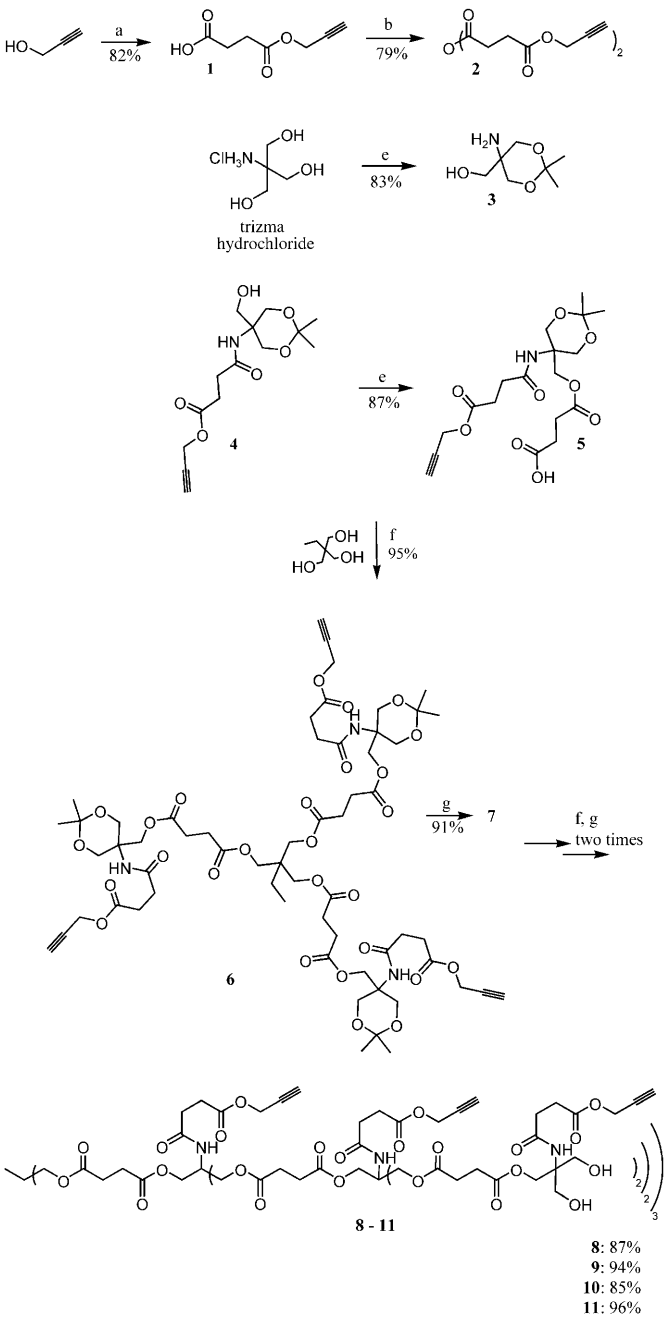
[**] We thank the Swedish Research Council (VR) Grant 2006-3617.

Supporting information for this article (including full experimental data, ^1H and ^{13}C NMR spectra, GPC traces, and details on materials and analytical techniques) is available on the WWW under http://dx.doi.org/10.1002/anie.200804987.

can undergo benign and efficient postfunctionalization reactions. These structures require more sophisticated synthetic protocols; the reports currently available include dendrimers containing phosphorus groups[9] or predetermined interior functionality,[10] or ones that utilize elaborate postfunctionalization strategies that require Ru catalysts, super bases, or strong acids.[11]

We present herein a benign synthetic methodology for the construction of a novel family of bifunctional dendrimers which comprise active internal and external functional groups (Figure 1 D). Our strategy is based on the recent accomplishments in chemoselective orthogonal reactions wherein traditional chemical reactions, such as esterification, amidation etc., are compatible with the copper(I)-catalyzed cycloaddition reaction between primary alkynes and azides (CuAAC; click reaction).[12] Initially, an AB_xC_y-type monomer was designed, where $x \geq 2$ and $y \geq 1$, and the A functional group can only react with the B functional group during dendritic growth. The C group will decorate the dendritic interior for postfunctionalization purposes. Trizma hydrochloride was identified as a building block for the preparation of the AB_xC_y monomer (Scheme 1). The resulting AB_2C monomer **5**, bearing one carboxylic group, one acetylene unit, and an acetonide-protected diol (A = COOH, B = OH, C = Acet; $x = 2$ and $y = 1$), was successfully obtained on a 30 gram scale.

A divergent growth approach from a trimethylol propane (TMP) core was chosen for the construction of the multifunctional dendrimers (Scheme 1). The synthetic methodology employed included the well-known esterification coupling reagent dicyclohexylcarbodiimide (DCC) for dendritic growth and the acidic Dowex resin for the activation/deprotection of the diols. In the first step, a 1.2 excess of the AB_xC_y monomer and DCC per active OH group was sufficient to obtain the first generation dendrimer **6**. The activation/deprotection by using acidic conditions was accomplished in greater than 90% yield, generating TMP-G1-$(Acet)_3$-OH_6 **7** with six activated hydroxy groups and three inert acetylene groups. Repetitive growth/activation reactions led to the bifunctional dendrimer TMP-G3-$(Acet)_{21}$-OH_{24} **11** with a total yield of 57% and an approximate molecular weight of 7300 g mol^{-1}. Moreover, the fully activated dendrimer was efficiently synthesized in six steps and decorated with 21 acetylene and 24 hydroxy groups, which can undergo robust postfunctionalizations. This synthesis is in contrast to the bifunctional dendrimers (Figure 1 B) which require a minimum of 16 steps to obtain 16+16 active groups.[4,5] To additionally illustrate the significance of our method, the newly developed dendrimers are compared to different dendritic scaffolds in a plot shown in Figure 2. The total number of functionalities (f_{tot}) is calculated by using the equation in Figure 2, where z is the number of functional groups in the core, x and y are the number of AB_xC_y monomers, and n represents the number of generations. Typically, a third generation dendrimer emanating from a trifunctional core has 24 functional groups, or 12+12 groups in the case of a peripheral bifunctional dendrimer. Our AB_2C-type dendrimer, for example, TMP-G3-$(Acet)_{21}$-OH_{24} **11**, has a total number of 45 functionlities (21 internal, 24 peripheral). For higher generations, the f_{tot} for AB_2C dendrimers



Scheme 1. Synthesis of bifunctional dendrimers comprising of acetylene groups on the interior and hydroxy groups on the periphery. a) Succinic anhydride, DMAP, CH_2Cl_2; b) DCC, CH_2Cl_2, 0 °C; c) 1. dimethoxypropane, DMF, *p*-TSA; 2. TEA; d) DMAP, CH_2Cl_2; e) succinic anhydride, DMAP, CH_2Cl_2; f) **5**, DCC, DMAP, DPTS, pyridine, CH_2Cl_2; f) acidic Dowex resin, MeOH. DMAP = 4-dimethylaminopyridine, DPTS = 4-(dimethylamino)pyridinium *p*-toluenesulfonate, TEA = triethylamine, *p*-TSA = toluene-*p*-sulfonic acid.

increases rapidly compared to peripheral bifunctional dendrimers.

All reactions were monitored by using MALDI-TOF analysis to ensure complete substitution of the growth sites, and the dendrimers were purified by using flash chromatography. The purity was analyzed by using NMR, GPC, and MALDI-TOF techniques. A typical NMR spectrum for the

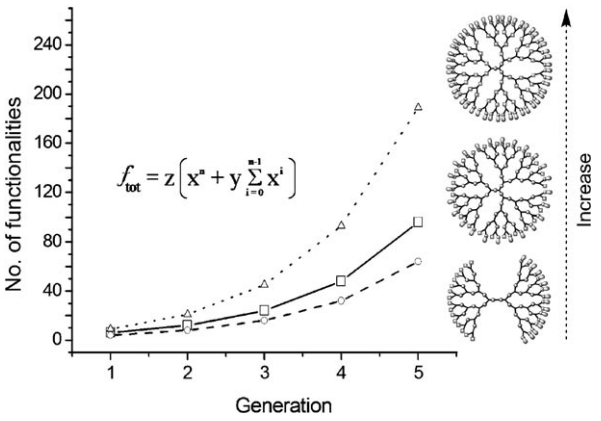


Figure 2. Increase in total number of functional groups. Comparison between functionality and the chemical composition of the dendritic architecture (three-armed core for dendrimers and two-armed core for the didendron). Bifunctional didendron (○); traditional dendrimer (□); bifunctional dendrimer having internal and peripheral functional groups (△).

third generation multifunctional dendrimer **10**, TMP-G3-$Acet_{21}$-Ac_{12}, can be seen in Figure 3. Additional evidence of the monodispersity is shown in the MALDI-TOF spectra (Figure 4). Interestingly, this technique was less suitable for the examination of higher generation multifunctionality dendrimers (beyond third generation). The increased complexity of higher generation dendritic frameworks having a

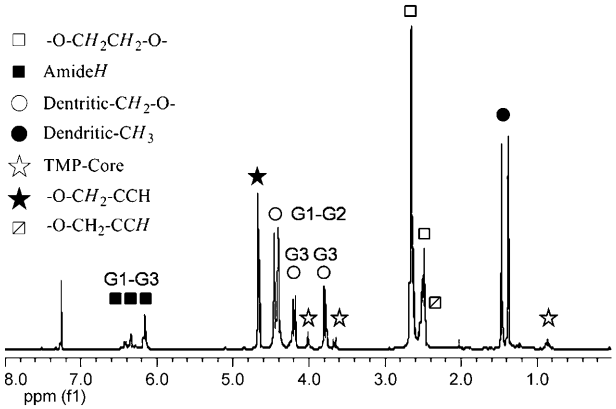


Figure 3. Representative ^{1}H NMR spectrum of an AB_2C dendrimer with internal acetylenes, TMP-G3-$Acet_{21}$-Ac_{12} **10.** G = generation.

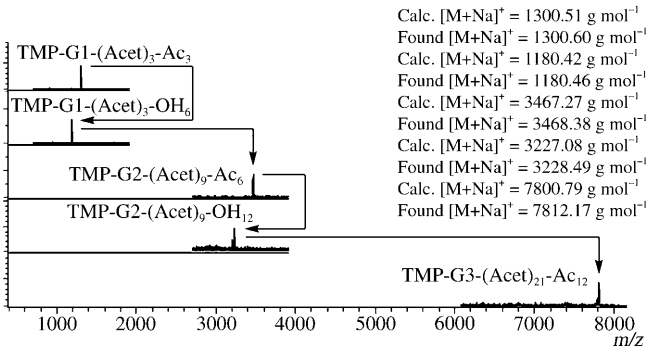
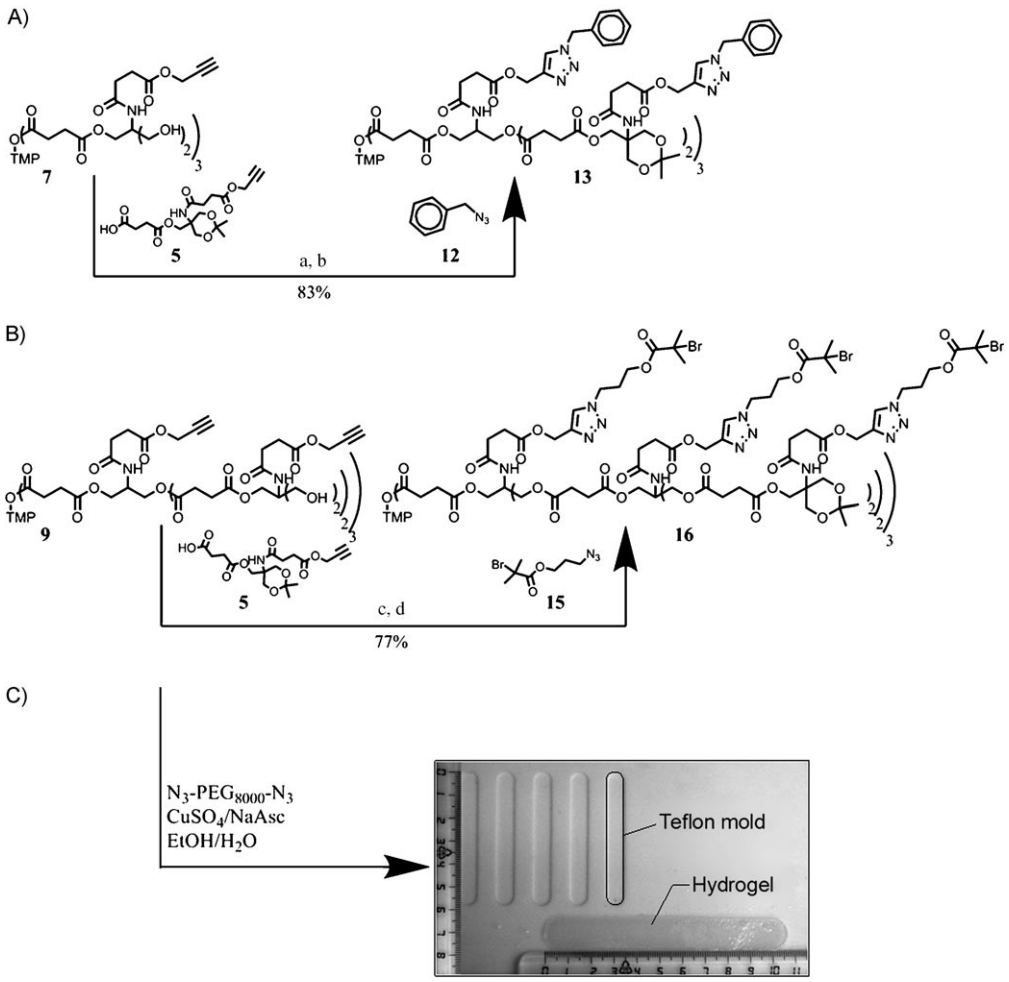


Figure 4. MALDI-TOF spectra of AB_2C dendrimers with internal acetylenes.

hydrophobic interior and a hydrophilic exterior was challenging for MALDI-TOF analysis and efforts are now underway to identify suitable matrices to confirm the purity of higher generation materials.

To provide insight into the chemoselective and orthogonal nature of these activated dendritic scaffolds, two different model postfunctionalization reactions were performed. A robust postfunctionalization strategy is the hallmark of synthetic efficiency and is essential for future scientific exploitation. Recent reports have described elegant examples of expedient methodologies in which the click reaction was compatible with chemical reactions including controlled radical polymerization, esterification, and amidation reactions.[13] Consequently, an in situ strategy was employed in both cases, targeting the efficient functionalization of the anchor groups on the interior and the groups on the exterior by using esterification and CuAAC reactions (Scheme 2). In the first model system, the first generation dendrimer TMP-G1-$(Acet)_1$-$(OH)_6$ **7** and AB_2C monomer **5** were dissolved in DMF and the esterification reagents (DCC/DMAP) were added (Scheme 2A). The reaction was monitored with MALDI-TOF methods, and upon completion of the reaction benzyl azide and the CuAAC reagents (CuBr/PMDETA) were added. During the postfunctionalization reaction dendrimer **7** underwent an in situ generation growth and the internal functionalization with benzylic groups generated TMP-G2-$(Benzyl)_9$-$(Ac)_6$ **13**, which was isolated after column chromatography. The second step involved the in situ reaction of the second generation dendrimer TMP-G2-$(Acet)_9$-$(OH)_{12}$ **9** with AB_2C monomer **5** and an azide functional initiator (3-azidopropyl 2-bromo-2-methylpropanoate, **15**), suitable for atom transfer radical polymerization (ATRP) techniques. By using a procedure similar to that used for the first model system, TMP-G3-$(Initiator\text{-}Br)_{21}$-$(Ac)_{12}$ **16** was effectively obtained, having an approximate molecular weight of 13000 g mol^{-1}, in 77% yield (Scheme 2B). This one-pot accelerated and benign postfunctionalization strategy opens the door for a highly tailored functionalization, which could deliver dendritic materials with increased sophistication as required for advanced applications, such as optical devices or drug delivery systems.

Dendritic materials are also candidates for multifunctional hydrogel crosslinkers. Hydrogels derived from AB_2C dendritic crosslinkers with anchored peptides can, for example, act as artificial extracellular matrices to allow accelerated interaction with body cells, promoting growth of new bone.[14] We believed that the AB_2C dendrimers, including biodegradable ester and amide bridges, would exhibit promising cytotoxic results for use in physiological environments. Consequently, TMP-G2-$(Acet)_9$-$(OH)_{12}$ **9** was assessed for potential cytotoxicity. An indirect contact test using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) staining on a MG-63 osteoblast cell line at both 4 mg mL^{-1} and 12 mg mL^{-1} was performed. Absorbance measurements to determine cell viability were performed at 0, 24, 48, and 72 hour time points. The dendrimer showed either no or low toxic effect at each of the two concentrations tested. The nontoxic nature of the dendrimer encouraged the study of their crosslinking ability in polyethylene glycol (PEG) based

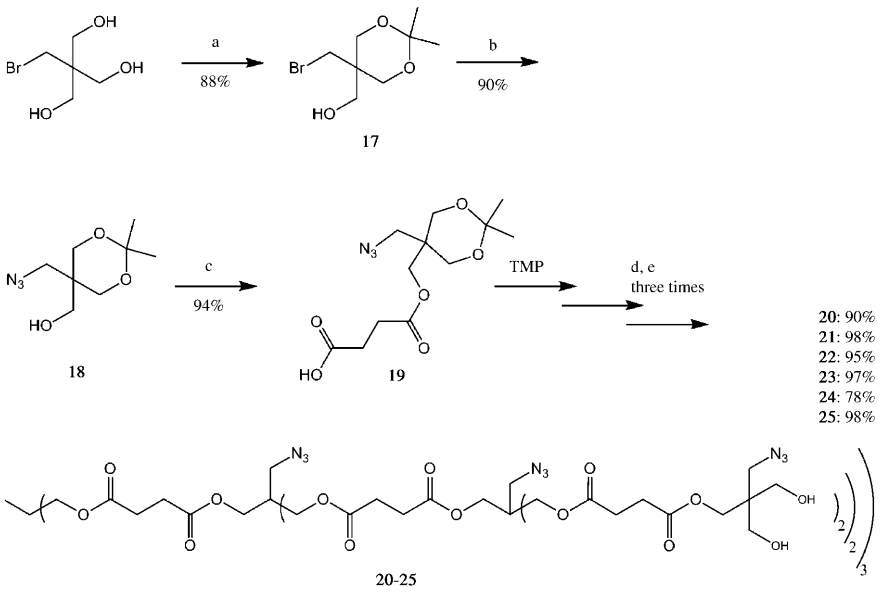


Scheme 2. One-pot in situ postfunctionalizations of AB_2C dendrimer interior and periphery. A) $2\times3\approx6$ acetonide end groups and $3+6\approx9$ benzylic groups: a) DCC, DMAP, DMF, RT; b) PMDETA, CuBr. B) $2\times2\times3\approx12$ acetonide end groups and $3+6+12\approx21$ initiators for ARTP: c) DCC, DMAP, THF, RT; d) $CuSO_4$, NaAsc, H_2O. C) PEG hydrogel **28** based on second generation dendritic crosslinker.

networks. By using a click-based hydrogel procedure,[15] an equimolar amount of the second generation dendrimer **9** and N_3-PEG_{8000}-N_3 **27** were dissolved in ethanol (50:50 mass %). $CuSO_4$/NaAsc (aq) was then added to the solution, and the reaction was run in a Teflon mold (Scheme 2C). Crosslinking was observed within 30 minutes, and after 24 hours the yellowish hydrogel was acquired. After extraction of the copper using aqueous EDTA (ethylenediaminetetracetic acid), the transparent hydrogel **28** having dendritic crosslinking junctions was obtained. The swollen hydrogel was found to contain 96% water and could be degradaded within 1 hour at pH 11 or 4 days at pH 4.

Next we made a second set of multifunctional dendrimers bearing internal azides instead of acetylene groups. 2-(Bromomethyl)-2-(hydroxymethyl)propane-1,3-diol was effectively converted into the AB_2C monomer **18** having one carboxylic group, one azide unit, and a protected diol (A = COOH, B = OH, C = N_3; $x=2$ and $y=1$). By adapting the same divergent growth/activation strategy, a third generation fully activated dendrimer, TMP-G3-$(N_3)_{21}$-OH_{24} **24**, was obtained in 54% yield and had an approximate molecular weight of 5400 g mol^{-1} (Scheme 3).

In contrast to the acetylenes, the azide groups can be exposed to UV light to release N_2 to generate imine and nitrene groups, which can additionally react within the dendritic framework.[16] From this perspective, multifunctional dendrimers equipped with internal azides can be exposed to UV light and theoretically undergo intramolecular crosslinking to generate collapsed nanoparticles or encapsulators, making them candidates for the delivery of agents or molecular sensors. An exciting report by Zimmermann and co-workers[17] reveals the intramolecular crosslinking of allyl decorated dendrimers using the Grubbs catalyst. Unfortunately, the reported nanoparticles are based on the toxic benzyl ether backbone. Therefore, a simple and alternative approach was investigated using the second generation dendrimer TMP-G2-$(N_3)_9$-$(Ac)_6$ **22**, which has nine internal azides. The dendrimer was diluted to 0.5 mg mL^{-1} in THF and divided equally (1 mL) into four quartz cuvettes. The solutions were exposed to UV irradiation with 1 to 4 pulses (scans) for 2 seconds at an intensity level of 0.537 J cm^{-2}. A sample from each solution was analyzed by GPC analysis to monitor the collapse efficiency (Figure 5). The decrease in the hydrodynamic volume from 11.7 Å to 9.6 Å confirmed the intramolecular collapse of the dendrimer into a more



Scheme 3. Synthesis of AB_2C dendrimers having internal azides. a) 1. Dimethoxypropane, *p*-TSA, acetone; b) NaN_3, DMSO, 80°C; c) succinic anhydride, DMAP, CH_2Cl_2; d) **19**, DCC, DMAP, DPTS, pyridine, CH_2Cl_2; e) acidic Dowex polymer resin, MeOH.

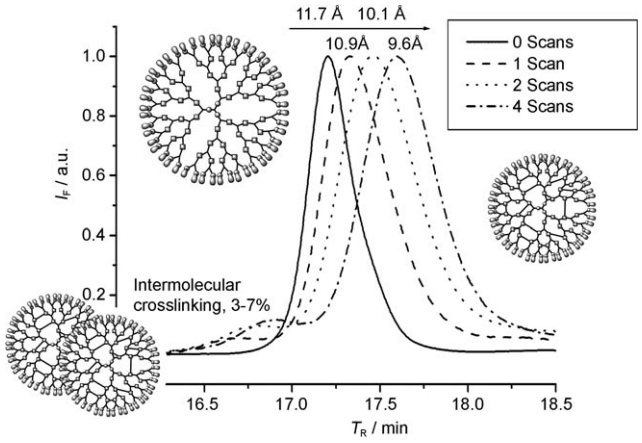


Figure 5. Simple UV-induced method for the intramolecular collapse of TMP-G2-$(N_3)_9$-$(Ac)_6$ **22** into dendritic nanoparticles.

confined structure. Furthermore, the loss of N_2 (−14 Da) was confirmed with MALDI-TOF analysis. A small fraction of intermolecular crosslinking (3–7%) was observed, which is believed to be concentration dependent.

In conclusion, we report herein a simple synthetic method for the construction of two bifunctional AB_2C dendrimers with acetylene/azides anchored within the interior and hydroxy groups on the periphery. Their postfunctionalization was successfully investigated utilizing an efficient one-pot protocol. In addition, the versatile nature of these dendrimers was explored in the fabrication of dendritic nanoparticles and hydrogels on the basis of dendritic crosslinkers. Additional studies are now being pursued to develop nanoparticles and hydrogels with increased complexity.

Received: October 13, 2008
Published online: December 29, 2008

Keywords: click chemistry · dendrimers · gels · nanoparticles · synthetic methods

[1] D. A. Tomalia, H. Baker, J. R. Dewald, M. Hall, G. Kallos, S. Martin, J. Ryder, P. Smith, *Polym. J.* **1985**, *17*, 117–132.
[2] G. R. Newkome, Z.-Q. Yao, G. R. Baker, V. K. Gupta, *J. Org. Chem.* **1985**, *50*, 2003–2004.
[3] C. J. Hawker, J. M. J. Frechet, *J. Am. Chem. Soc.* **1990**, *112*, 7638–7647.
[4] a) E. R. Gillies, J. M. J. Frechet, *J. Am. Chem. Soc.* **2002**, *124*, 14137–14146; b) C. C. Lee, E. R. Gillies, M. E. Fox, S. J. Guillaudeu, J. M. J. Fréchet, E. E. Dy, F. C. Szoka, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 16649–16654.
[5] P. Wu, M. Malkoch, J. N. Hunt, R. Vestberg, E. Kaltgrad, M. G. Finn, V. V. Fokin, K. B. Sharpless, C. J. Hawker, *Chem. Commun.* **2005**, 5775–5777.
[6] V. Maraval, R. Laurent, B. Donnadieu, M. Mauzac, A. M. Caminade, J. P. Majoral, *J. Am. Chem. Soc.* **2000**, *122*, 2499–2511.
[7] a) T. Kawaguchi, K. L. Walker, C. L. Wilkins, J. S. Moore, *J. Am. Chem. Soc.* **1995**, *117*, 2159–2165; b) F. Zeng, S. C. Zimmerman, *J. Am. Chem. Soc.* **1996**, *118*, 5326–5327; c) R. Haag, A. Sunder, J. F. Stumbe, *J. Am. Chem. Soc.* **2000**, *122*, 2954–2955; d) L. Brauge, G. Magro, A. M. Caminade, J. P. Majoral, *J. Am. Chem. Soc.* **2001**, *123*, 6698–6699; e) P. Antoni, D. Nyström, C. J. Hawker; A. Hult, M. Malkoch, *Chem. Commun.* **2007**, 2249–2251; A. Hult, M. Malkoch, *Chem. Commun.* **2007**, 2249–2251.
[8] A. P. Goodwin, S. S. Lam, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2007**, *129*, 6994–6995.
[9] M. L. Lartigue, M. Slany, A. M. Caminade, J. P. Majoral, *Chem. Eur. J.* **1996**, *2*, 1417–1426.
[10] W. R. Dichtel, S. Hecht, J. M. J. Fréchet, *Org. Lett.* **2005**, *7*, 4451–4454.
[11] a) C. O. Liang, J. M. J. Fréchet, *Macromolecules* **2005**, *38*, 6276–6284; b) C. Galliot, C. Larre, A.-M. Caminade, J.-P. Majoral, *Science* **1997**, *277*, 1981–1984; c) L. Lochmann, K. L. Wooley, P. T. Ivanova, J. M. J. Fréchet, *J. Am. Chem. Soc.* **1993**, *115*, 7043–7044.
[12] a) R. Huisgen, *1,3-Dipolar Cycloaddition—Introduction, Survey, Mechanism*, Wiley, Hoboken, **1984**, pp. 1–176; b) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021; c) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; d) C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.
[13] P. Lundberg, C. J. Hawker, A. Hult, M. Malkoch, *Macromol. Rapid Commun.* **2008**, *29*, 998–1015.
[14] M. P. Lutolf, F. E. Weber, H. G. Schmoekel, J. C. Schense, T. Kohler, R. Müller, J. A. Hubbel, *Nature* **2003**, *421*, 513–518.
[15] M. Malkoch, R. Vestberg, N. Gupta, L. Mespouille, P. Dubois, A. F. Mason, J. L. Hedrick, Q. Liao, C. W. Frank, K. Kingsbury, C. J. Hawker, *Chem. Commun.* **2006**, 2774–2776.
[16] P- Ling. C. A. Wight, *J. Phys. Chem. B.* **1997**, *101*, 2126–2131.
[17] L. G. Schultz, Y. Zhao, S. C. Zimmermann, *Angew. Chem.* **2001**, *113*, 2016–2020; *Angew. Chem. Int. Ed.* **2001**, *40*, 1962–1966.

