Biocompatible Nanotubes

DOI: 10.1002/anie.200705363

Biocompatible Carbon Nanotubes Generated by Functionalization with Glycodendrimers**

*Peng Wu, Xing Chen, Nancy Hu, Un Chong Tam, Ola Blixt, Alex Zettl, and Carolyn R. Bertozzi**

The structural, mechanical, electrical, and optical properties of single-walled carbon nanotubes (SWNTs) have stimulated considerable interest in their biological applications.[1–3] SWNTs have been employed for biosensing,[4] imaging,[5] intracellular delivery,[6] and cancer cell targeting.[7,8] However, expanded use of SWNTs in living systems will require strategies to diminish their cytotoxicity.[9–12] Thus, surface modifications that mitigate the toxicity of SWNTs while simultaneously enabling specific biological recognition are highly sought after.[8,13–17]

A promising avenue we have recently explored is to coat SWNTs with synthetic glycopolymers that mimic the glycoproteins found on cell surfaces.[16,17] We demonstrated that lipid-terminated poly(methyl vinyl ketone)-based glycopolymers can coat carbon nanotube (CNT) surfaces and promote their binding to cells through receptor–ligand interactions.[16,17] Importantly, the modified CNTs were nontoxic to cultured cells. These findings were tempered, however, by the irregular surface and nonuniform thickness of the CNT coating, which reflected the high polydispersities (>1.7) of the polymers employed.[18] Such surface heterogeneity might undermine the use of glycopolymer-coated CNTs as sensors of protein binding.

Herein, we report the use of glycodendrimers as homogeneous bioactive coatings for CNTs. In addition to various biomedical applications,[19–21] dendrimers have been used to functionalize CNTs with photoactive groups,[22] to improve their solubility,[23] and to introduce sites for metal detection.[24] Their branched architectures and high density of peripheral functional groups have prompted several research groups to explore glycodendrimers as mimics of cell-surface glycans.[25–27] Inspired by these examples as well as recent breakthroughs in dendrimer synthesis using click chemistry,[28–30] we developed a new class of bifunctional glycodendrimers based on 2,2-bis(hydroxymethyl)propionic acid, a biocompatible building block. As depicted in Scheme 1, the dendrimers (**1**) have peripheral carbohydrate units and a pyrene tail capable of binding SWNT surfaces through π–π interaction. Their geometry is reminiscent of the multiantenna N-linked glycans that populate eukaryotic cell surfaces.

The synthesis employed the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction previously used by Sharpless and Hawker to prepare diverse dendritic structures.[30,31] In our work, the CuAAC reaction allowed for chemoselective ligation of azide-functionalized pyrene and glycan moieties to the alkyne-functionalized focal point and chain ends of a dendritic scaffold, respectively (Scheme 1). The synthetic glycans, each with an azidoethyl aglycone,[32] remained unprotected during glycodendrimer assembly.

Applying this methodology, we prepared a panel of [G-2] (**2a–c**) and [G-3] (**1a–c**) glycodendrimers with a variety of carbohydrate structures in near quantitative yield (see reference [33] for nomenclature key). The pyrene tail **3** was conjugated to the focal point of **4**[25] by CuAAC, and the resulting dendrimer **5** was further coupled with pent-4-ynoic anhydride (**6**) to introduce additional alkyne groups to the periphery (Scheme 1). The resulting dendrimer **7** was then reacted with a 2-azidoethyl mono- or disaccharide (**8a–c**) using CuAAC to furnish the desired [G-3] glycodendrimers **1a–c**. Analysis of the dendrimers by NMR spectroscopy and MALDI-TOF mass spectrometry confirmed that the structures were homogeneous (see the Supporting Information).

The glycodendrimers were adsorbed onto SWNTs by ultrasonication in aqueous solution (see the Supporting Information for experimental details), which resulted in complete solubilization (Figure 1a, I–VI). The suspensions of glycodendrimer-functionalized SWNTs were stable for several months in water, whereas the unfunctionalized

[*] Prof. C. R. Bertozzi
Departments of Chemistry and Molecular and Cell Biology and Howard Hughes Medical Institute, University of California
and
The Molecular Foundry, Lawrence Berkeley National Laboratory
Berkeley, CA 94720 (USA)
Fax: (+1) 510-643-2628
E-mail: crb@berkeley.edu

P. Wu,[+] X. Chen,[+] N. Hu, U. C. Tam
Department of Chemistry, University of California
Berkeley, CA 94720 (USA)

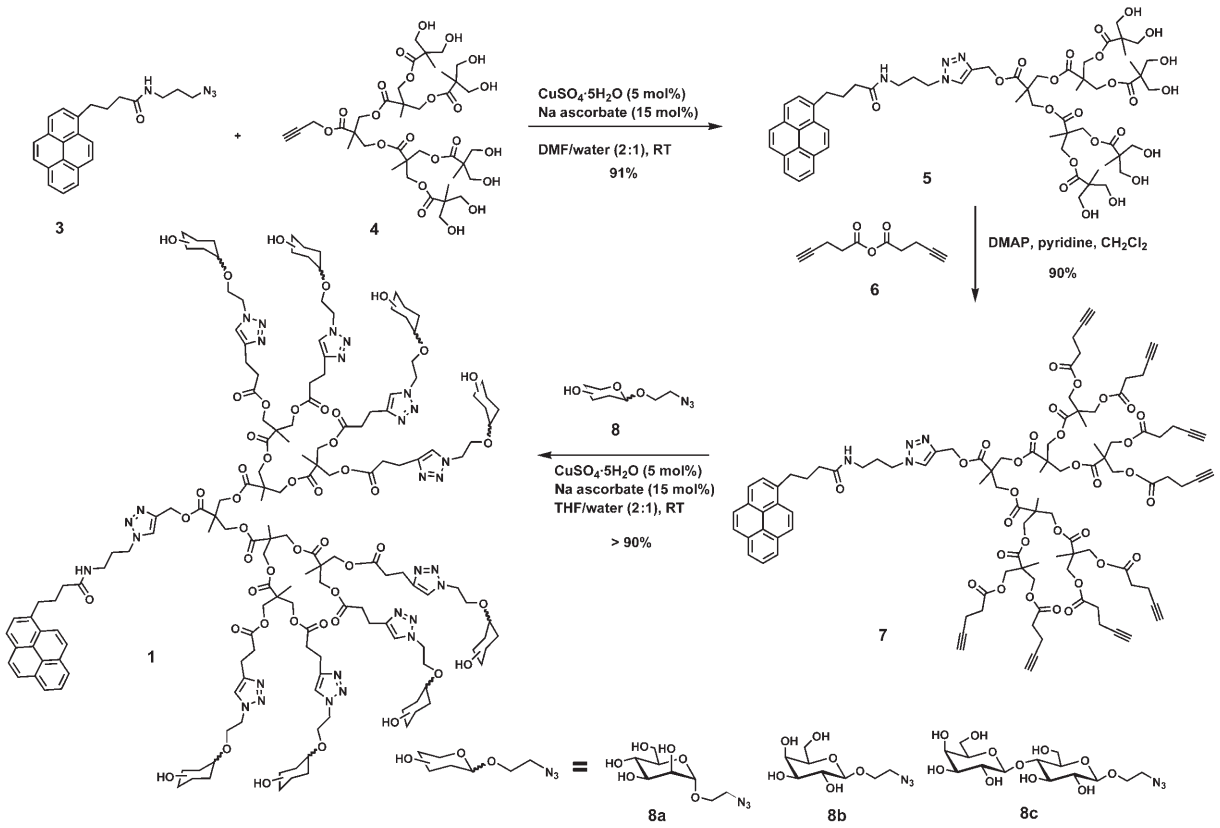
O. Blixt
Carbohydrate Synthesis and Protein Expression Core D
Consortium for Functional Glycomics
The Scripps Research Institute, La Jolla, CA 92037 (USA)

Prof. A. Zettl
Department of Physics, University of California
and
Materials Sciences Division, Lawrence Berkeley National Laboratory
Berkeley, CA 94720 (USA)
E-mail: azettl@socrates.berkeley.edu

[+] These authors contributed equally to the work.

[**] This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Materials Sciences, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098, within the Interfacing Nanostructures Initiative and NIH (K99M080585-01). Portions of this work were performed at the Molecular Foundry, Lawrence Berkeley National Laboratory, which is supported by the Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. **9c** was provided by Consortium for functional Glycomics.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Synthesis of glycodendrimers: generation 3 [G-3] dendrimers are depicted; generation 2 [G-2] dendrimers were synthesized by a similar process. DMAP = 4-(dimethylamino)pyridine.

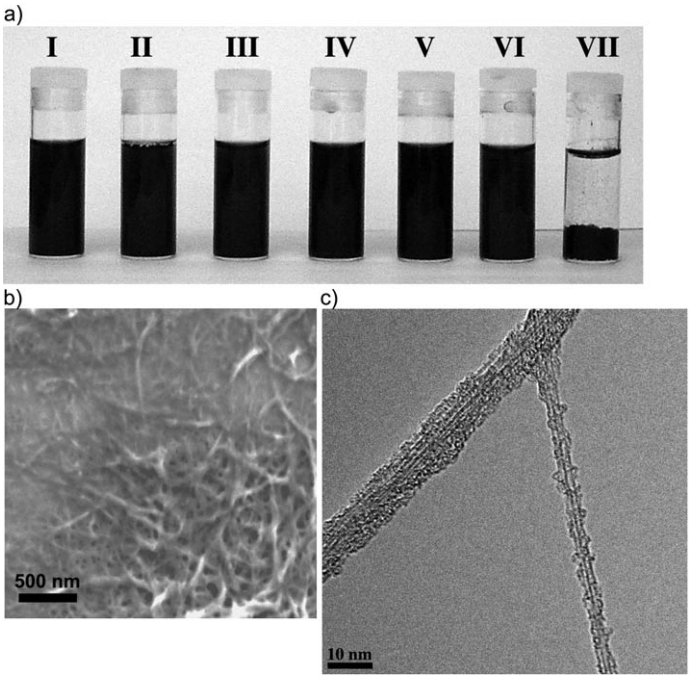


Figure 1. a) Vials containing SWNT suspensions. [G-2] Man-SWNTs (I), [G-2] Gal-SWNTs (II), [G-2] Lac-SWNTs (III), [G-3] Man-SWNTs (IV), [G-3] Gal-SWNTs (V), and [G-3] Lac-SWNTs (VI) formed stable suspensions in water for more than three months, while as-produced SWNTs (VII) precipitated from water within several hours. b) SEM and c) TEM images of [G-3] Man-SWNTs. The glycodendrimer coating can be visualized as the density surrounding the SWNT.

SWNTs precipitated within one hour (Figure 1a, VII). The [G-2] glycodendrimer-coated SWNTs precipitated slowly after several months, most likely the result of interactions among coated nanotubes enabled by a relatively sparse hydrophilic coating. Accordingly, the precipitates were readily redissolved by ultrasonication. Control glycodendrimers lacking the pyrene moiety had no solubilizing effect on SWNTs (data not shown). Scanning electron microscopy (SEM; Figure 1b) and transmission electron microscopy (TEM; Figure 1c) analysis revealed small bundles and individual SWNTs coated entirely with a thin uniform layer of glycodendrimers. These images contrast markedly with the thick heterogeneous coatings observed with CNT-bound glycopolymers.[16]

Specific binding of SWNT-bound glycodendrimers to receptors is critical for sensing and targeting applications. We probed this capability with a panel of fluorescein isothiocyanate (FITC)-conjugated lectins: *Canavalia ensiformis* agglutinin (Con A), *Arachis hypogaea* agglutinin (PNA), and *Psophocarpus tetragonolobus* agglutinin (PTA), which recognize α-mannose, lactose, and β-galactose, respectively. SWNTs coated with different glycodendrimers were incubated with FITC-conjugated lectins then dialyzed to remove unbound protein and analyzed by fluorescence spectroscopy. Significant fluorescence was observed for Con A-treated [G-3] Man-SWNTs, [33] whereas only background fluorescence was

observed for PTA- or PNA-treated [G-3] Man-SWNTs (Figure 2a). Similarly, [G-3] Gal-SWNTs bound to FITC-conjugated PTA, but not to PNA or Con A. As the non-reducing terminal monosaccharide in Lac is Gal, [G-3] Lac-SWNTs were recognized by both PNA and PTA, but not Con A. Similar results were obtained from parallel studies using SWNTs coated with [G-2] glycodendrimers (data not shown).

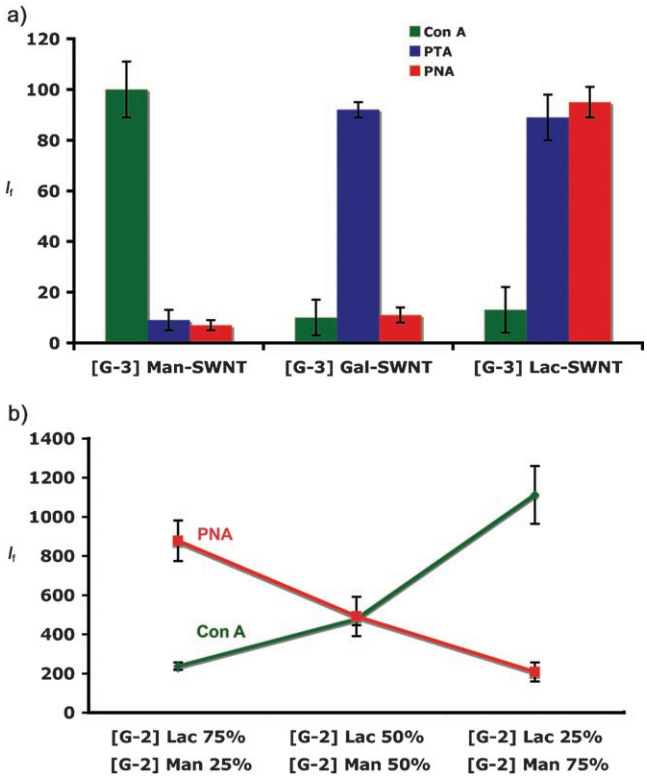


Figure 2. a) Lectin binding to glycodendrimer-coated SWNTs. Con A and PNA exhibit specific binding to [G-3] Man-SWNTs and [G-3] Lac-SWNTs, respectively. PTA binds to both [G-3] Gal-SWNTs and [G-3] Lac-SWNTs. Error bars: standard deviation for three replicate experiments. b) Modulation of lectin binding to SWNTs cofunctionalized with two different glycodendrimers. SWNTs coated with [G-2] Lac and [G-2] Man at various ratios were incubated with a 1:1 (molar ratio) mixture of Texas Red-conjugated PNA and FITC-conjugated Con A. The intensities of Texas Red and FITC emission paralleled the ratio of [G-2] Lac and [G-2] Man components in the SWNT coating, respectively. Error bars: standard deviation for three replicate experiments. I_f: fluorescence intensity.

To more accurately mirror the complexity of biological glycoconjugates, which typically display multiple glycan epitopes, we functionalized SWNTs with a mixture of [G-2] Man (**2a**) and [G-2] Lac (**2c**) at various ratios. After dialysis to remove unbound glycodendrimers, the coated SWNTs were incubated with a 1:1 (molar ratio) mixture of Texas Red-conjugated PNA and FITC-conjugated Con A. As shown in Figure 2b, the SWNT-associated Texas Red fluorescence intensity decreased while the FITC fluorescence increased along with increasing percentage of [G-2] Man in the SWNT coating. Thus, multiple epitopes displayed on SWNTs can bind simultaneously to discrete proteins.

We hypothesized that after binding to the [G-2] Man-SWNTs, the tetravalent lectin Con A[34] would still possess open sites for further complexation with Man residues on cell-surface glycans. The carbohydrate–lectin interaction could thereby promote SWNT binding to cell surfaces. Toward this end, [G-2] Man-SWNTs were first treated with FITC-conjugated Con A (FITC-Con A). Following dialysis, the functionalized SWNTs were incubated with Chinese hamster ovary (CHO) cells. Fluorescence microscopy analysis revealed specific binding of modified SWNTs to the cell membrane (Figure 3). As a control, we performed the same experiment using SWNTs coated with the [G-2] Gal (**2c**) dendrimer. In this case, no fluorescent labeling of the cells was observed (data not shown). By contrast, [G-2] Gal-SWNTs and [G-2] Lac-SWNTs labeled the CHO cells robustly when PTA and PNA were used as cross-linkers, respectively (data not shown).

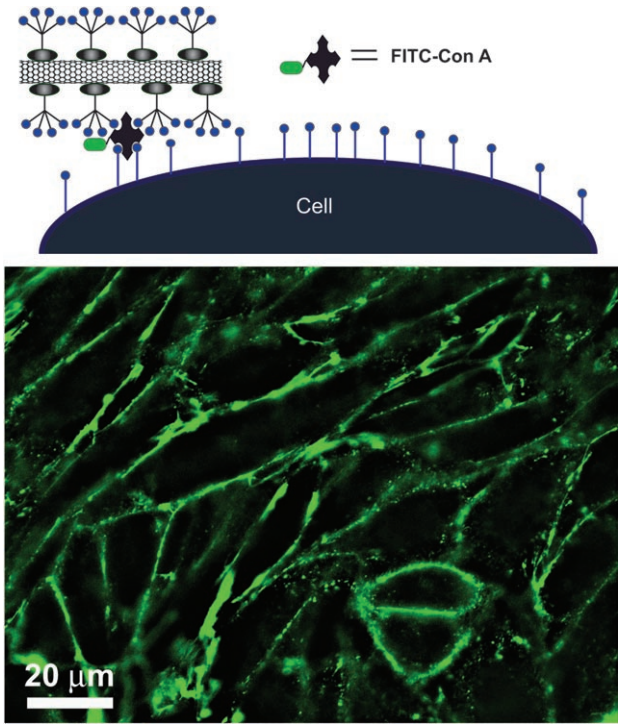


Figure 3. Top: The multivalent lectin Con A can bind glycodendrimer-coated SWNTs to cells. Bottom: Fluorescence micrograph of CHO cells labeled with complexes of [G-2] Man-SWNT and FITC-Con A.

Finally, to evaluate their cytotoxicity we co-cultured [G-2] and [G-3] glycodendrimer-coated SWNTs (100 $\mu g\,mL^{-1}$) with HEK293 cells for four days. In control experiments, the cells were incubated with unmodified SWNTs or with media alone. Viable cells (Trypan Blue assay) were counted each day. Cells cultured with glycodendrimer-coated SWNTs proliferated at the same rate as cells grown in the absence of SWNTs (Figure 4). By contrast, unmodified SWNTs greatly hampered the growth of HEK293 cells. Notably, the relatively thin coating produced by glycodendrimers appears to passivate

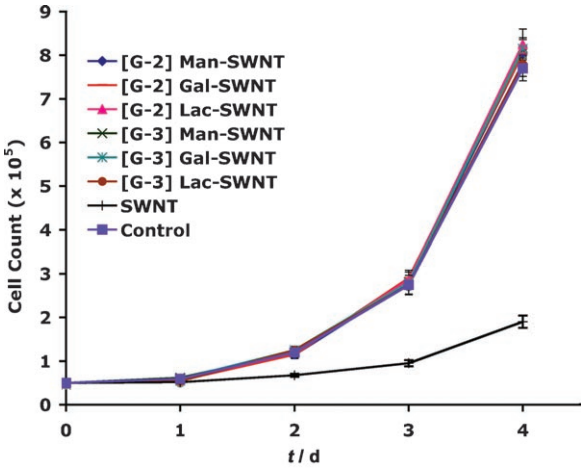


Figure 4. Effects of glycodendrimer-coated SWNTs on the proliferation of HEK293 cells. Live cells were quantified by the Trypan Blue dye exclusion method. Control cells were grown in the absence of SWNTs. Error bars: standard deviation for three replicates.

SWNTs against cytotoxicity as effectively as much thicker glycopolymer coatings.[17]

In conclusion, glycodendrimers can function as homogeneous bioactive coatings for SWNTs that also mitigate their cytotoxicity. The synthetic method used to construct the glycodendrimers can be readily adapted to ligands for other receptor interactions. Future applications include biosensors for carbohydrate-binding proteins and delivery agents that target specific cell-surface receptors.

Received: November 22, 2007
Published online: May 28, 2008

Keywords: biomimetic synthesis · carbon · dendrimers · glycoconjugates · nanotubes

[1] Y. Lin, S. Taylor, H. P. Li, K. A. S. Fernando, L. W. Qu, W. Wang, L. R. Gu, B. Zhou, Y. P. Sun, *J. Mater. Chem.* **2004**, *14*, 527.
[2] D. Tasis, N. Tagmatarchis, A. Bianco, M. Prato, *Chem. Rev.* **2006**, *106*, 1105.
[3] R. S. Kane, A. D. Stroock, *Biotechnol. Prog.* **2007**, *23*, 316.
[4] A. Star, E. Tu, J. Niemann, J. C. P. Gabriel, C. S. Joiner, C. Valcke, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 921.
[5] S. S. Wong, E. Joselevich, A. T. Woolley, C. L. Cheung, C. M. Lieber, *Nature* **1998**, *394*, 52.
[6] K. Kostarelos, L. Lacerda, G. Pastorin, W. Wu, S. Wieckowski, J. Luangsivilay, S. Godefroy, D. Pantarotto, J. P. Briand, S. Muller, M. Prato, A. Bianco, *Nat. Nanotechnol.* **2007**, *2*, 108.
[7] N. W. Shi Kam, M. O'Connell, J. A. Wisdom, H. J. Dai, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11600.
[8] Z. Liu, W. B. Cai, L. N. He, N. Nakayama, K. Chen, X. M. Sun, X. Y. Chen, H. J. Dai, *Nat. Nanotechnol.* **2007**, *2*, 47.
[9] V. L. Colvin, *Nat. Biotechnol.* **2003**, *21*, 1166.
[10] D. X. Cui, F. R. Tian, C. S. Ozkan, M. Wang, H. J. Gao, *Toxicol. Lett.* **2005**, *155*, 73.
[11] G. Jia, H. F. Wang, L. Yan, X. Wang, R. J. Pei, T. Yan, Y. L. Zhao, X. B. Guo, *Environ. Sci. Technol.* **2005**, *39*, 1378.
[12] A. Magrez, S. Kasas, V. Salicio, N. Pasquier, J. W. Seo, M. Celio, S. Catsicas, B. Schwaller, L. Forro, *Nano Lett.* **2006**, *6*, 1121.
[13] M. S. Arnold, M. O. Guler, M. C. Hersam, S. I. Stupp, *Langmuir* **2005**, *21*, 4705.
[14] C. M. Sayes, F. Liang, J. L. Hudson, J. Mendez, W. H. Guo, J. M. Beach, V. C. Moore, C. D. Doyle, J. L. West, W. E. Billups, K. D. Ausman, V. L. Colvin, *Toxicol. Lett.* **2006**, *161*, 135.
[15] H. Dumortier, S. Lacotte, G. Pastorin, R. Marega, W. Wu, D. Bonifazi, J. P. Briand, M. Prato, S. Muller, A. Bianco, *Nano Lett.* **2006**, *6*, 1522.
[16] X. Chen, G. S. Lee, A. Zettl, C. R. Bertozzi, *Angew. Chem.* **2004**, *116*, 6237; *Angew. Chem. Int. Ed.* **2004**, *43*, 6111.
[17] X. Chen, U. C. Tam, J. L. Czlapinski, G. S. Lee, D. Rabuka, A. Zettl, C. R. Bertozzi, *J. Am. Chem. Soc.* **2006**, *128*, 6292.
[18] D. Rabuka, R. Parthasarathy, G. S. Lee, X. Chen, J. T. Groves, C. R. Bertozzi, *J. Am. Chem. Soc.* **2007**, *129*, 5462.
[19] C. C. Lee, J. A. MacKay, J. M. J. Frechet, F. C. Szoka, *Nat. Biotechnol.* **2005**, *23*, 1517.
[20] Y. Kim, S. C. Zimmerman, *Curr. Opin. Chem. Biol.* **1998**, *2*, 733.
[21] S. Svenson, D. A. Tomalia, *Adv. Drug Delivery Rev.* **2005**, *57*, 2106.
[22] S. Campidelli, C. Sooambar, E. L. Diz, C. Ehli, D. M. Guldi, M. Prato, *J. Am. Chem. Soc.* **2006**, *128*, 12544.
[23] Y. P. Sun, W. J. Huang, Y. Lin, K. F. Fu, A. Kitaygorodskiy, L. A. Riddle, Y. J. Yu, D. L. Carroll, *Chem. Mater.* **2001**, *13*, 2864.
[24] M. Holzinger, J. Abraha, P. Whelan, R. Graupner, L. Ley, F. Hennrich, M. Kappes, A. Hirsch, *J. Am. Chem. Soc.* **2003**, *125*, 8566.
[25] 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.
[26] W. B. Turnbull, J. F. Stoddart, *Rev. Mol. Biotechnol.* **2002**, *90*, 231.
[27] M. J. Cloninger, *Curr. Opin. Chem. Biol.* **2002**, *6*, 742.
[28] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004.
[29] J.-F. Lutz, *Angew. Chem.* **2007**, *119*, 1036; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018.
[30] P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Frechet, K. B. Sharpless, V. V. Fokin, *Angew. Chem.* **2004**, *116*, 4018; *Angew. Chem. Int. Ed.* **2004**, *43*, 3928.
[31] C. J. Hawker, K. L. Wooley, *Science* **2005**, *309*, 1200.
[32] F. Fazio, M. C. Bryan, O. Blixt, J. C. Paulson, C. H. Wong, *J. Am. Chem. Soc.* **2002**, *124*, 14397.
[33] Glycodendrimer nomenclature: Man = α-D-mannopyranoside, Gal = β-D-galactopyranoside, Lac = 1,4-(β-D-galactopyranosyl)-β-D-glucopyranoside; [G-2] denotes generation 2 and [G-3] denotes generation 3. [G-2] Man, [G-2] Gal, [G-2] Lac, [G-3] Man, [G-3] Gal, and [G-3] Lac denote the corresponding glycodendrimers.
[34] G. N. Reeke, J. W. Becker, G. M. Edelman, *J. Biol. Chem.* **1975**, *250*, 1525.

