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Dendrimer Synthesis and Functionalization by Click Chemistry for Biomedical Applications

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8.1 Introduction

Dendrimer-based platforms have achieved increasing attention for use in biomedical applications including, but not limited to, targeted drug delivery, imaging and transfection. Interest in dendrimers can be attributed to their unique branching structure that results in exceptionally high degrees of monodispersity as compared with other polymeric materials. Furthermore, well-defined terminal groups allow the conjugation of multiple functional molecules. Dendrimer systems with multiple copies of targeting ligands enhance the interaction of targeting molecules with cell membrane receptors due to multivalent binding.[1] While the concept of preferential targeting is not limited to dendrimer platforms, the ability to create dendrimers that mimic the size and shape of human proteins makes the technology an ideal choice for many therapeutic and diagnostic applications. The dendrimer's nanometer size enables efficient diffusion across the vascular endothelium and directs internalization into cancer cells, and facilitates rapid renal clearance of these molecules from the blood stream.

The most widely used dendrimers in biomedical applications are poly(amidoamine) (PAMAM) dendrimers. The polyamide backbone helps the macromolecule maintain water solubility and minimizes immunogenicity. PAMAM dendrimers exhibit little toxicity if the surface amines have been neutralized or modified (Figure 8.1).[2–7]

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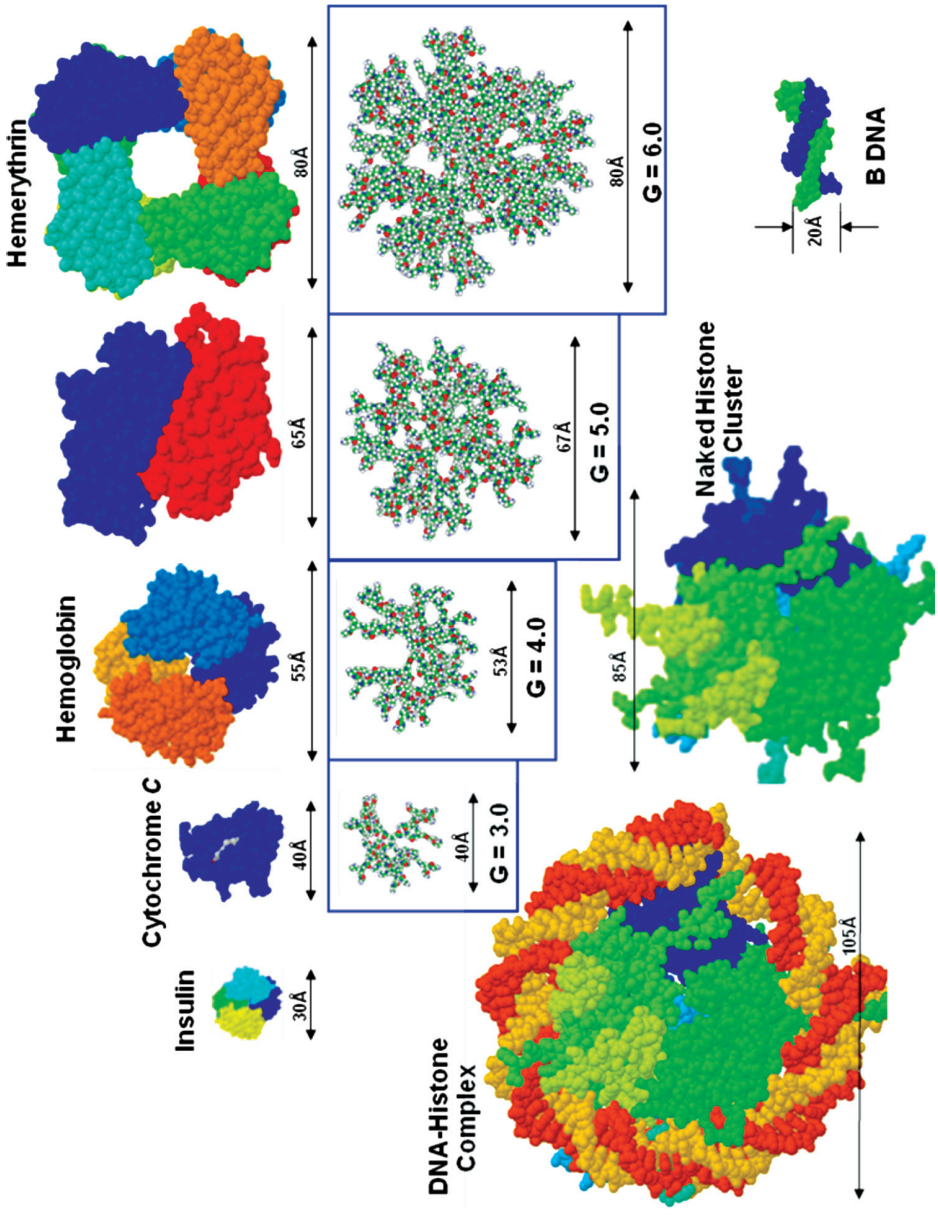

Figure 8.1 *Comparison of absolute size of PAMAM dendrimers and proteins.*

The PAMAM platform has been successfully used as a scaffold for the attachment of targeting molecules including antibodies,[8–12] peptides,[13] T-antigens[14–16] and folic acid.[17–26] The targeting ligands anchor the dendrimers to locations where receptors are expressed on cell surfaces. Dendrimers have also been used to carry and solubilize therapeutic molecules, such as methotrexate.[19,22,23,27,28] Targeted dendrimer–drug conjugates deliver a higher dose specifically to tumor cells while avoiding normal cells, thus avoiding the systemic toxicity of current therapeutics. PAMAM dendrimers with folate and methotrexate have been shown to specifically bind to carcinoma KB cells expressing high levels of the folate receptor.[23] Targeted chemotherapy with dendrimers showed 10 times the efficacy and decreased toxicity compared with standard chemotherapy with free methotrexate during *in vivo* studies. Phase I clinical trials for this cancer therapy are planned (Figure 8.2).

One can imagine dendrimer scaffolds being modified to create a vast library of targeted therapeutics, each tailored to address the properties and overexpressed receptors of an individual target cell. Dendrimer treatment could be personalized after a screening to determine which therapeutic would have the greatest effect.

Several dendrimer-based products are under development for the treatment of a variety of diseases. Starpharma is testing a topical polylysine dendrimer-based microbicide, VivaGel™, for the prevention of HIV transmission and other sexually transmitted diseases during intercourse. SuperFect®, developed by Qiagen, is an activated dendrimer used for gene transfection in a broad range of cell lines. Dendrimers have also been used as diagnostic tools. Gadomer-17, a polylysine dendrimer functionalized with gadolinium chelates, from Schering AG is used as MRI contrast agent. The US Army Research Laboratory developed Alert Ticket™ as a dendrimer-based diagnostic for anthrax detection. Stratus® CS, has been commercialized by Dade Behring for the rapid diagnosis of heart attacks by acting as a biosensor for cardiac markers.

Despite the promise and successes of dendrimer platforms, the field's ability to provide materials for biomedical applications has been slowed by synthetic challenges in producing mono-dispersed bulk dendrimers. PAMAM dendrimer synthesis can lead to undesired side reactions causing defect structures, and these defect structures propagate as the dendrimer grows with each additional generation.[29,30] Therefore, although PAMAM dendrimers maintain a very low PDI, maintaining batch-to-batch reproducibility can be challenging. When these polymers are conjugated with multiple targeting and therapeutic molecules, the heterogeneity of the conjugates is magnified. Unfortunately, this makes the characterization of these materials difficult and the material itself must be reproducible and consistent if one hopes to eventual administer these agents to human subjects. Other dendrimers based on different subunit structures may avoid side reactions and ease reproducibility concerns, but are poor choices for many biomedical applications because of poor solubility at physiological conditions and/or cytotoxicity. In addition, creating multifunctional dendrimers for therapeutic applications currently involves multiple, step-wise conjugations that are time-consuming and hard to reproduce. New synthetic approaches will be needed to produce consistent materials. In addition, when producing patient-specific drugs ('personalized medicine') some method of parallel synthesis must be achieved to produce many combinations of drug and targeting ligands that allow for individualized therapeutics.

'Click chemistry' is an approach that could enhance the synthesis of dendrimeric structures. There have been several examples involving the synthesis and conjugation of

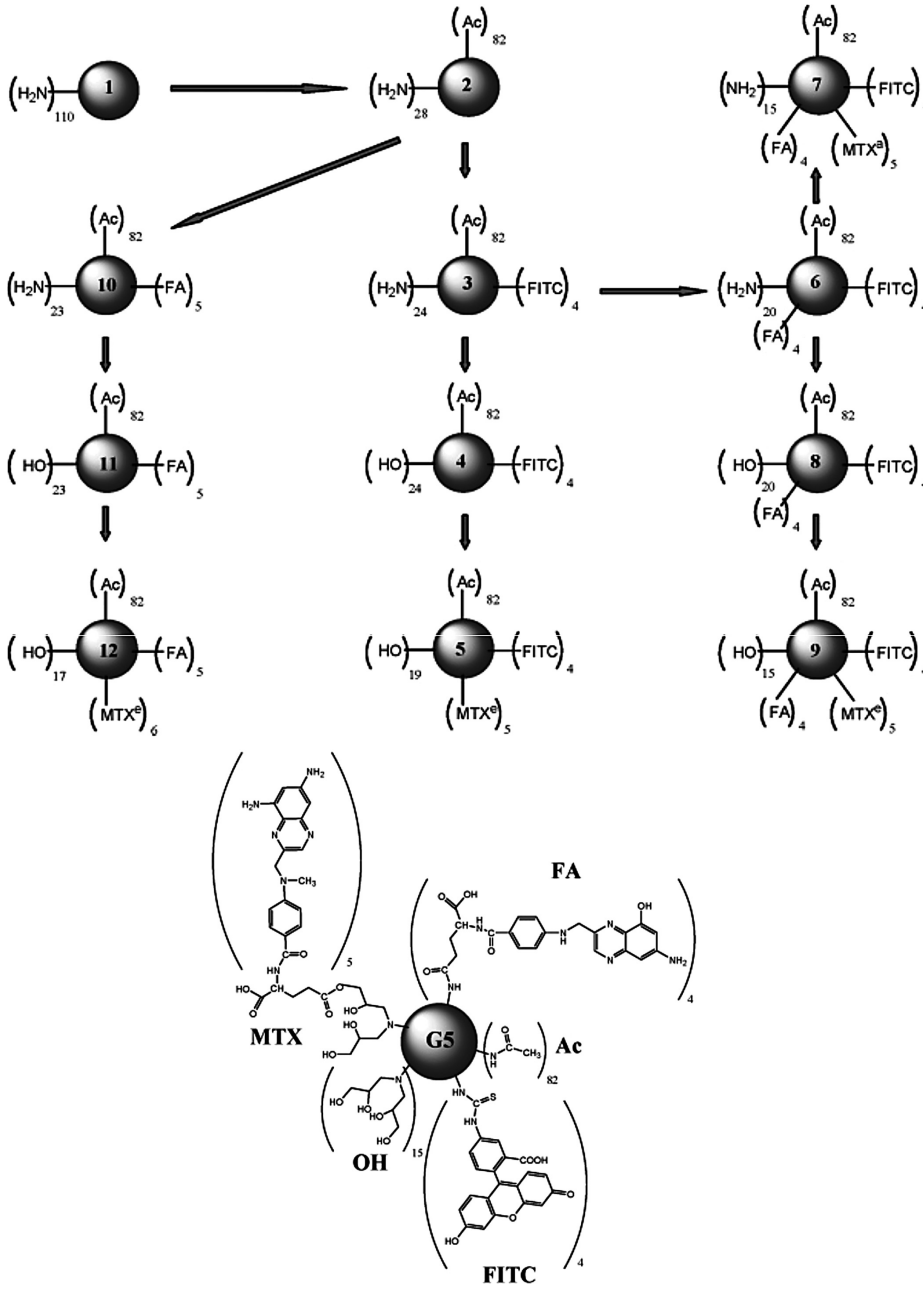

Figure 8.2 *Multifunctional device on a single dendrimer. Reprinted with permission from ref.[22]. Copyright 2005 American Chemical Society.*

dendrimers using click chemistry since Sharpless and co-workers popularized the copper(I)-catalyzed 1,3-dipolar cycloaddition.[31] Researchers hope to use click chemistry to obtain greater control over the synthesis of the dendrimer platforms by minimizing defect structures and reducing the need for purification. Others use click chemistry as a means to control the conjugation of desired functionalities.[32–39] The orthoganality of click chemistry provides a means to avoid incompatible conjugation reactions, reduce product heterogeneity and potentially add specific numbers of functional moieties.

8.2 Dendrimer Synthesis

Dendrimers have been synthesized by two methods: divergent and convergent.[40] These methods both create dendritic macromolecules through repeated growth and activation reactions, and each additional cycle adds an additional dendrimer 'generation'. Divergent synthesis proceeds radially from the core. Convergent synthesis proceeds from the surface inward, where molecular building blocks form dendrons which are then joined together at their unique focal points. Convergent synthesis typically yields dendrimers of higher purity and reproducibility compared with divergent methods; however, there is greater difficulty in producing larger generation dendrimers using convergent methods due to increasing steric constraints on the synthesis.[41]

Traditional synthetic approaches to dendrimer synthesis, whether convergent or divergent, are inefficient in their time and materials requirements (Figure 8.3). Some of the dendrimers that are commercially available may not be of appropriate quality for biomedical purposes, including PAMAM, phosphorous-based, polypropylenimine, polylysine, polyester and 2,2-bis(methylol)propionic acid (bis-MPA) dendrimers. A highly-trained synthetic chemist may require months to prepare dendrimers in significant quantities and quality adequate for bioconjugation. New synthetic methods which accelerate and simplify dendrimer production may be required in order to increase the amount and type of dendrimers necessary for many pharmaceutical applications. Attractive methods must produce dendrimer in good yields, minimize defect population distribution and display tolerance of functional groups and reaction conditions while cutting back on reaction and purification time. To accomplish these goals, various click chemistry reactions have been used to synthesize dendrimers by both divergent and convergent methods.

8.2.1 Divergent Synthesis

Click chemistry has been used on multiple occasions to divergently synthesize dendrimers. The copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) is used during the growth step, where the dendrimer is reacted with an alkyne or azide monomer unit.[43] These reactions have been performed at quantitative yields with minimal workup. Unfortunately, dendrimers divergently synthesized via the CuAAC have yet to be tested in biological systems. A possible issue with these molecules is the large number of heteroaromatics in these dendrimers, which may prohibit the material from being soluble in many physiological conditions.

An exciting development by Hawker and colleagues employs thiol-ene click chemistry to divergently synthesize dendrimers.[44] The thiol–alkene reaction is thought to be a robust,

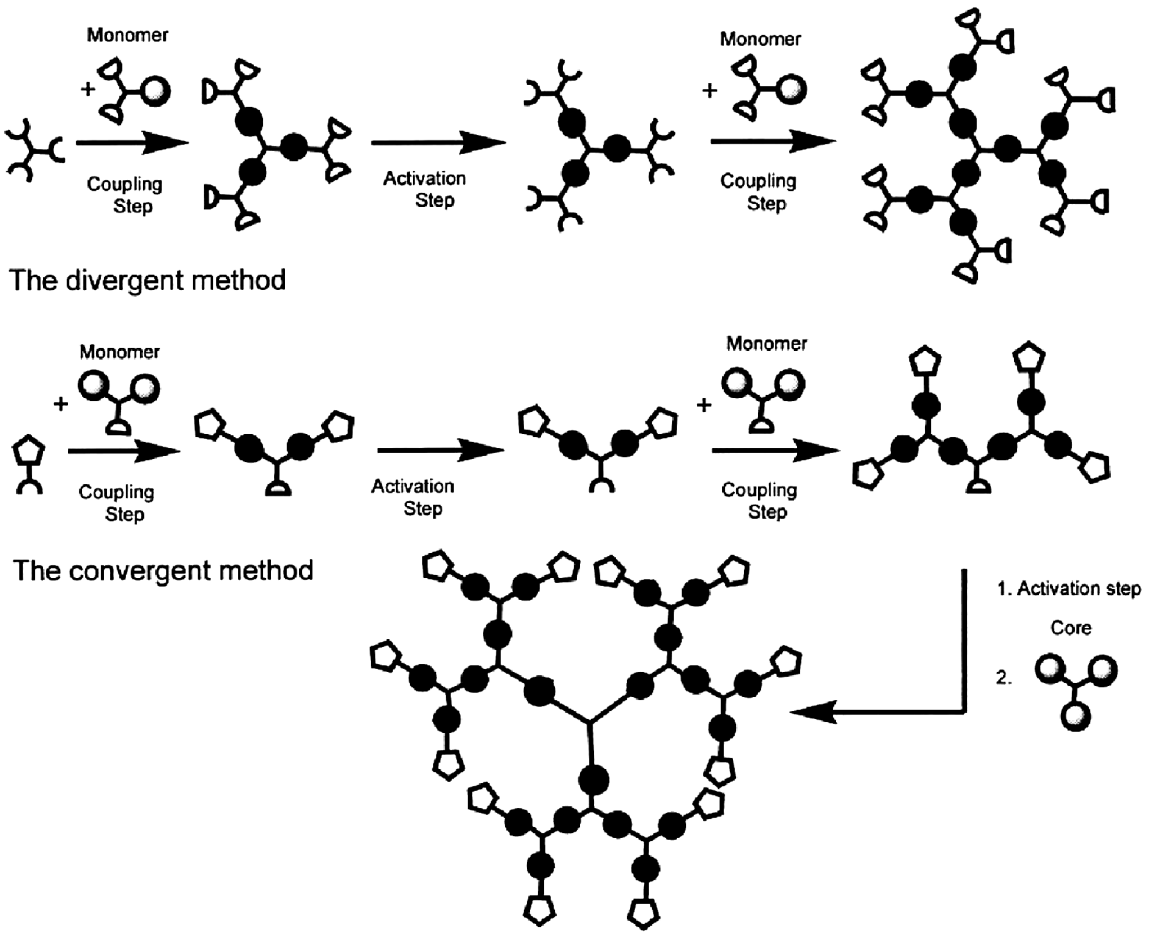

Figure 8.3 *Divergent and convergent dendrimer synthesis pathways. Reprinted with permission from ref.[42]. Copyright 2005 John Wiley and Sons, Inc.*

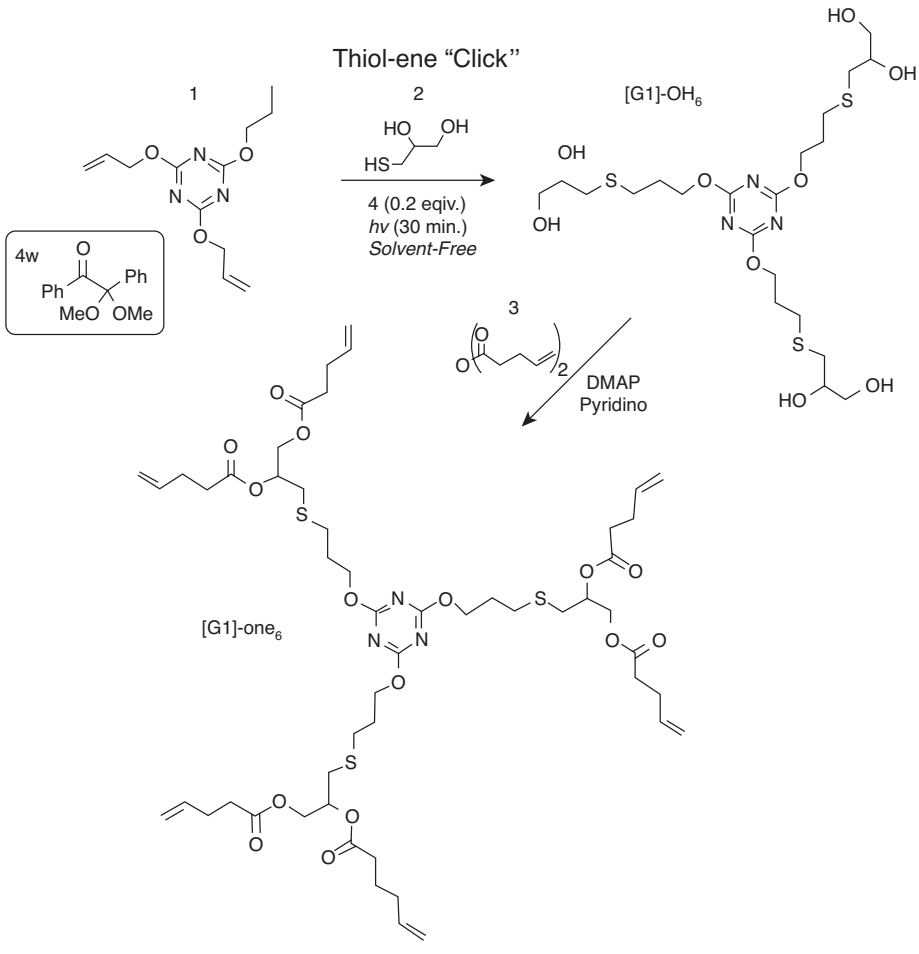

Figure 8.4 *Dendrimer synthesized using thiol-ene chemistry. Reprinted with permission from ref.[44]. Copyright 2008 American Chemical Society.*

clean reaction that avoids the use of a metal catalyst as with some other 'click' approaches. In addition, this chemistry can be performed without a solvent while maintaining the specificity properties of click chemistry. Generation 4 poly(thio-ether) dendrimers were constructed using this approach and functionalized with various surface groups using this chemistry. No biological studies have yet been published for this material, but the approach may open the door for the creation of a larger number of dendritic platforms for biomedical applications (Figure 8.4).

8.2.2 Convergent Synthesis

Convergent synthesis of dendrimers has been achieved for both symmetrical[32,34,45–50] and asymmetrical dendrimers.[33,45,49,50] A click reaction can be employed as the final step to combine dendrons with each other or to a core after traditional approaches are used to synthesize the dendrons.[33,45–52] CuAAC[48,50] and Diels–Alder[53] reactions have both been

employed to make dendrimers. In 2007, Haridas reported the design of peptide dendrimers with varied cores using CuAAC.[34] The group proposes conjugating biologically relevant molecules to the amino groups of the dendrimer. Wysozogrodzka applied the convergent method to polyglycerol dendrons of varying generation to create core-shell structures.[32] This approach successfully solubilized different hydrophobic cores and the hydrophobic dye Nile Red. Wysozogrodzka showed the amount of Nile Red solubilized depended on core size and generation of the polyglycerol dendrons (Figure 8.5).

The application of click chemistry to convergent synthesis also has the potential to be used for a mix-and-match approach to creating multifunctional macromolecules based on dendrimers. Uniquely functionalized dendrons can be selected from a library and combined to create functionalized devices for specific applications. Proof-of-concept reactions have been performed by Lee and co-workers on methyl ester-terminated, half-generation PAMAM dendrons to create symmetrical and unsymmetrical dendrimers.[49–51] This methodology has the potential to be adapted to functionalized full-generation dendrons that are soluble in physiological conditions. The same group has successfully coupled dendrons with different backbones to create diblock co-dendron devices.[51] Wu and co-workers synthesized unsymmetrical bis-MPA dendrimers, which were then functionalized to create a bifunctional, targeted device.[33]

In general, studies have indicated that the yield of the click reaction decreases with increasing dendron generation. It is proposed that steric effects, the dendron branches backfolding over the focal point, cause the decreased yield (Figure 8.6).[45,48–50]

8.3 Dendrimer Functionalization

The orthoganality and efficiency of click chemistry makes it an attractive means to functionalize dendritic macromolecules. Click chemistry has repeatedly been shown to be an efficient way to functionalize dendrimers with either carbohydrates,[35] peptides[36] or solubilizing linkers[37] for biomedical applications. This has provided increased potency and multivalent targeting because of the dendritic platform.

Wu and co-workers successfully produced a multifunctional bis-MPA dendron with targeted and imaging units using the 1,3-dipolar cycloaddition.[33] The dendron-bound mannose was reported to show a 240-fold greater potency in hemagglutination *vs* monomeric mannose. Dijkgraaf showed that radiolabeled tetrameric RGD dendrimers had enhanced uptake in both *in vitro* binding assays and $\alpha_V\beta_3$ integrin expressing tumors *in vivo* when compared with dimeric and monomeric RGD controls. This increased avidity was accomplished without altering the specificity of binding.[36] In 2006, Fernandez-Megia surface conjugated unprotected carbohydrates to produce PEG-lyated glycodendrimers for potential applications in exploring carbohydrate–receptor interactions or for targeted drug delivery (Figures 8.7 and 8.8).

Urbani recently combined atom transfer radical polymerization and click chemistry to design third-generation polymeric dendrimers with a peripheral generational layer that could be selectively cleaved off from the second-generation via basic hydrolysis of the dendrimers ester groups.[39] This platform can be used for the applications requiring a slow and controlled release of its peripheral layer (Figure 8.9).

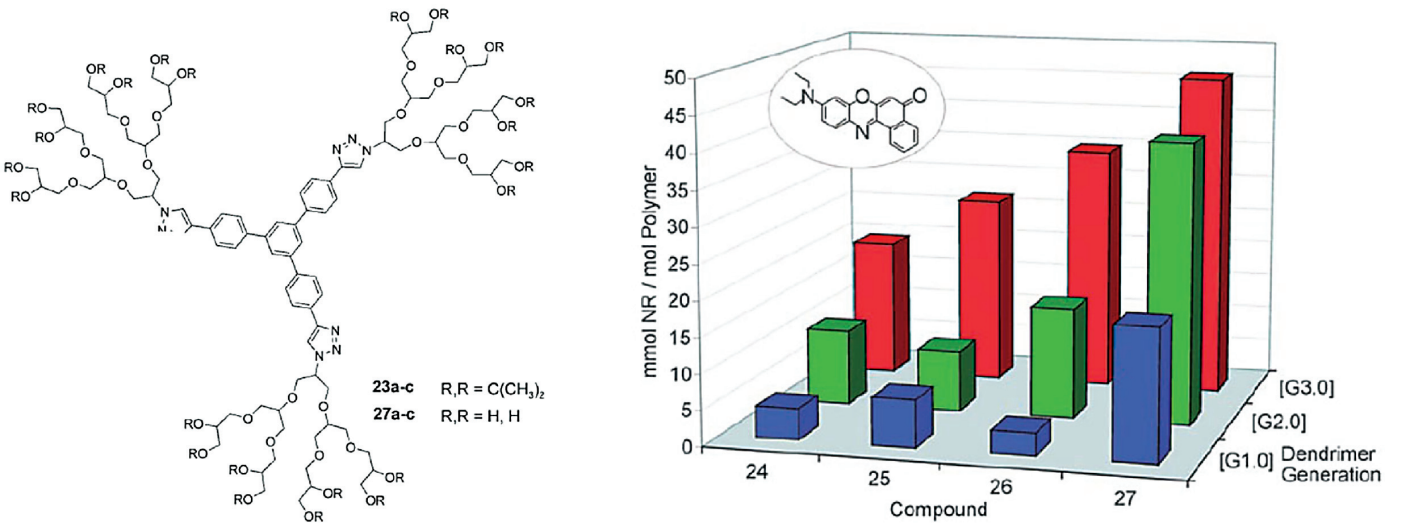

Figure 8.5 *Convergent synthesis of polyglycerol dendrimers and effect of generation on Nile Red solubilization. Reprinted with permission from ref.*[32]*. Copyright 2008 Wiley-VCH.*

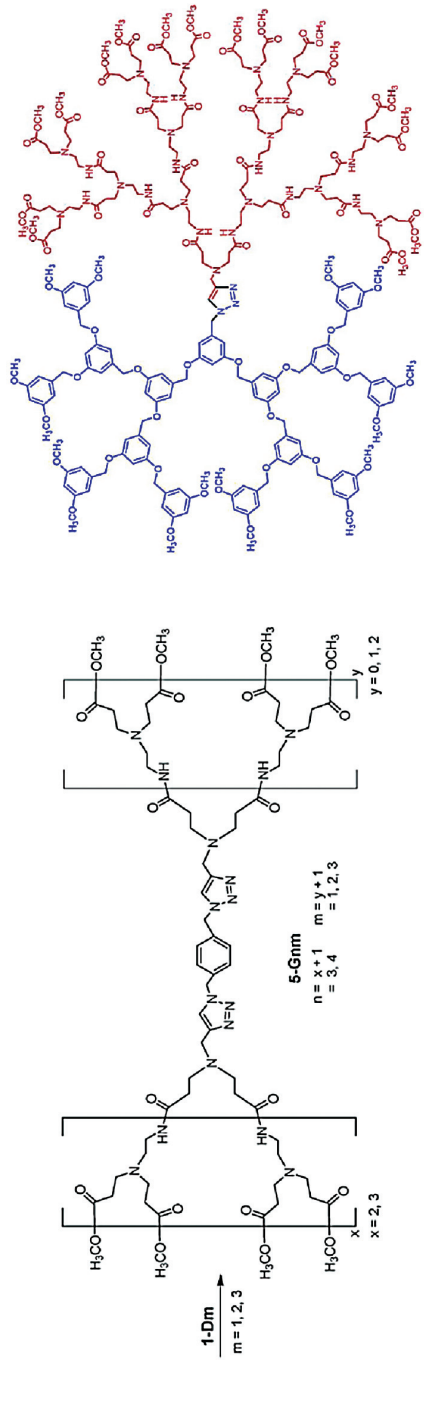

Figure 8.6 *Convergent synthesis of asymmetrical dendrimers. Reprinted with permission from ref.*[51]*. Copyright 2006 American Chemical Society.*

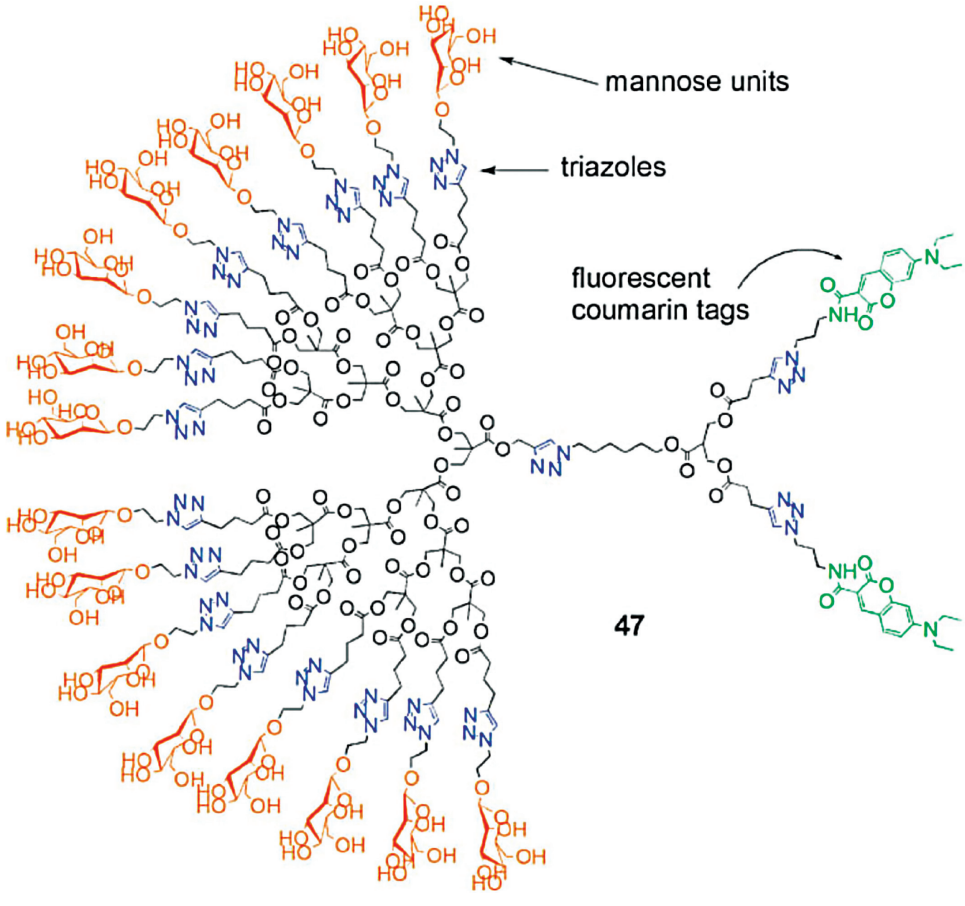

Figure 8.7 *Bifunctional bis-MPA dendrimer with 16 mannose units and 2 coumarin units. Reprinted with permission from ref.[33]. Copyright 2005 The Royal Society of Chemistry.*

CuAAC reactions have been used as an efficient means to functionalize solid supports or surfaces with dendritic materials. For example, Ortega-Munoz and co-workers conjugated glyco-dendrimers to a silica core to create a bio-selective affinity chromatography matrix for potential applications for the inmobilization of other biomolecules.[38] Pohl showed the functionalization of cellulose surfaces with propargyl–PAMAM dendrons via CuAAC.[54] A model enzyme, glucose oxidase, was covalently attached to the dendron and protein attachment was shown. These biofunctionalized surfaces have the potential to be used for various sensor, catalytic and delivery applications.

Click reactions have also been exploited for their orthoganality and neighboring group tolerance. When stoichiochemistry is critical, click chemistry can be used as a valuable tool to specifically couple single functionalities. The most obvious example is having a unique 'clickable' focal point on a dendron (Figure 8.10).

Work has been done by the Weck group to apply unique reactive sites to dendrimers as well. Polyamide dendrimers have been designed with a single azide or alkyne moiety on

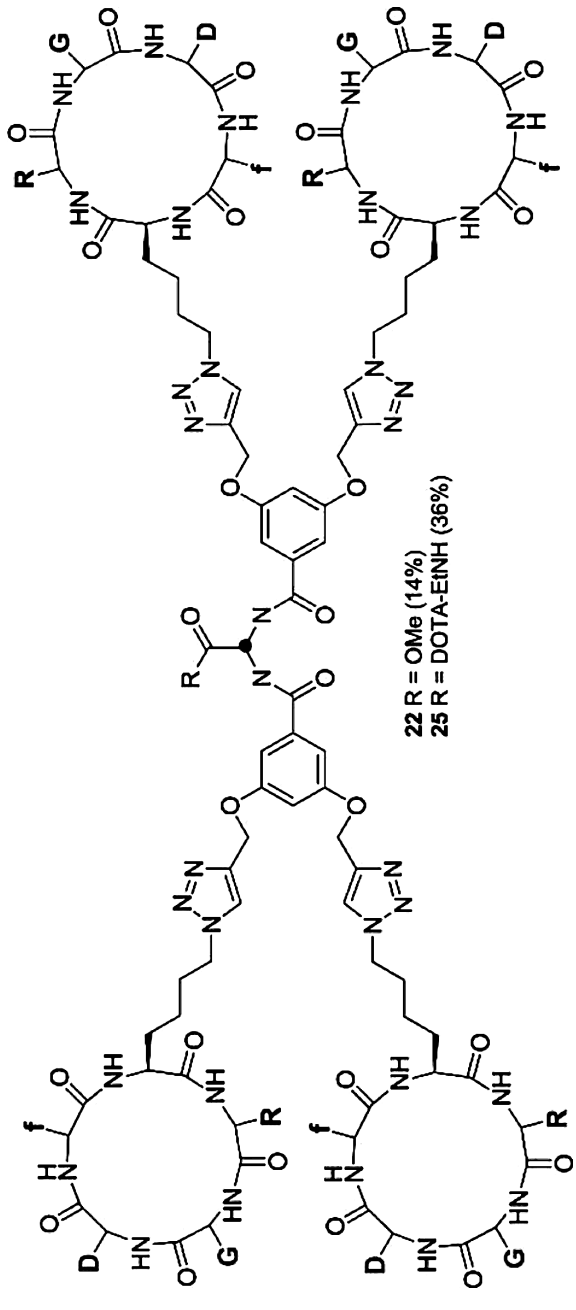

Figure 8.8 *Tetrameric RGD-dendrimer synthesized via CuAAC. Reprinted with permission from ref.*[36]*. Copyright 2007 The Royal Society of Chemistry.*

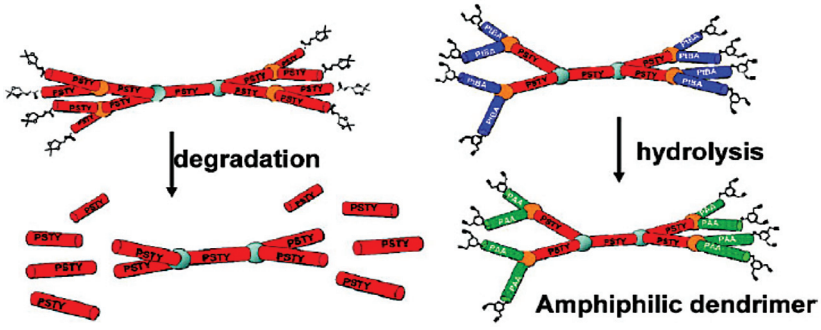

Figure 8.9 *Generation 3 polymeric dendrimer with cleavable periphery. Reprinted with permission from ref.[39]. Copyright 2008 American Chemical Society.*

the surface.[55,56] One can imagine using similar scaffolds when product distributions must be avoided or to create dumbbell structures without fear of forming insoluble networks (Figure 8.11).

8.4 Conclusions and Future Directions

The unique branched structure of dendrimers offers many benefits, from multivalent binding to increased load capacity. Yet standard techniques in dendrimer chemistry are laborious and the costs can be prohibitive. The inability of commercial suppliers to reproducibility

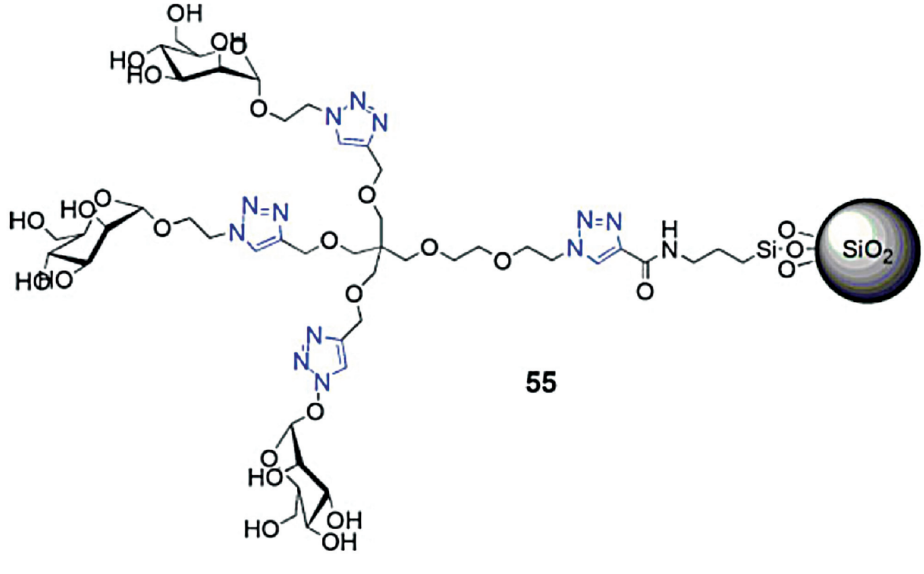

Figure 8.10 *Glyco-dendrimer conjugated to a silica core for affinity chromatography. Reprinted with permission from ref.[38]. Copyright 2006 Wiley-VCH.*

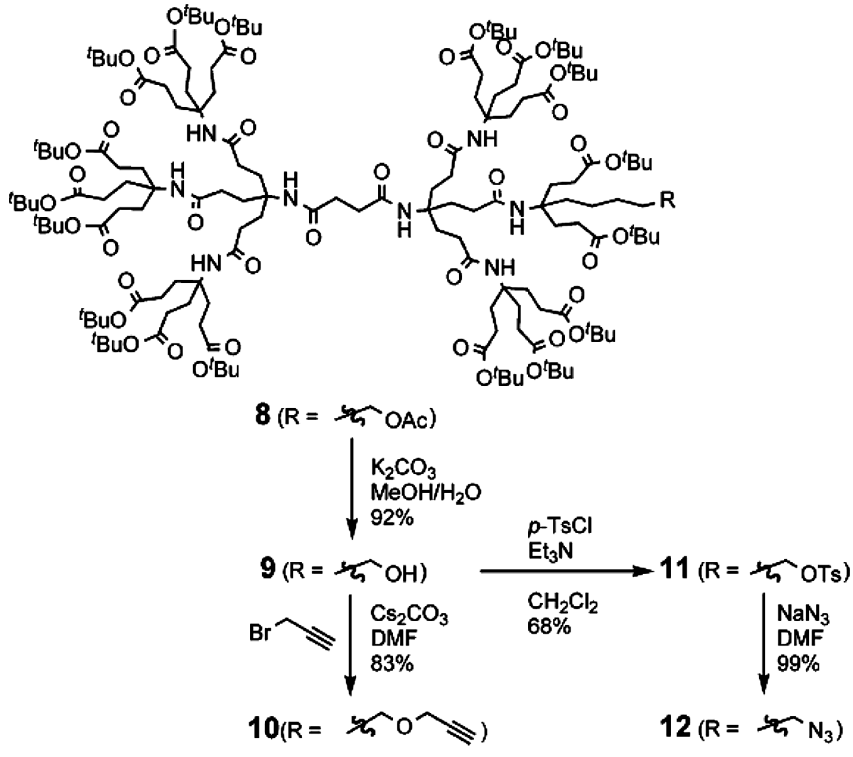

Figure 8.11 *Polyamide dendrimer with single orthogonal surface reactive site. Reprinted with permission from ref.[56]. Copyright 2007 American Chemical Society.*

minimize defect structures in highly biocompatible PAMAM has slowed their progress in reaching therapeutic trials. Many alternative dendrimers either suffer from the same problems or lack biocompatible and nontoxic properties. For dendrimers to become reliable and affordable scaffolds for medical use as targeted therapeutics, detection agents or diagnostics, their production must become more efficient and reproducible on a large scale. Expanding the use of click chemistry in dendrimer science will most likely continue to facilitate these goals.

Specifically, click chemistry has been applied to address dendrimer synthetic concerns. New shorter-term developments will probably come via functionalization of dendrimers, where reaction orthoganality and simpler purification will speed up and increase the production of new devices. Longer-term solutions needed include using click reactions to create methods of dendrimer synthesis. Click-type reactions have been applied to dendrimer synthesis, but the majority have used repeated triazole-based click reactions which typically produce dendrimers that are not soluble in physiological conditions. The use of the thiol-ene reaction shows that click methodologies can be developed that maintain biological applicability. Dendrimer synthesis will dramatically improve as additional clean, efficient reactions are developed to create non-immunogenetic, biofunctional scaffolds.

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