Dendrimer Synthesis

DOI: 10.1002/anie.201003968

A Synthetic "Tour de Force": Well-Defined Multivalent and Multimodal Dendritic Structures for Biomedical Applications

*Lars Röglin, Edith H. M. Lempens, and E. W. Meijer**

bioorganic chemistry · chemoselective ligations · dendrimers · macromolecular chemistry · multivalency

***D**endrimers have several unique properties that make them attractive scaffolds for use in biomedical applications. To date, multivalent and multimodal dendritic structures have been synthesized predominantly by statistical modification of peripheral groups. However, the potential application of such probes in patients demands well-defined and monodisperse materials that have unique structures. Current progress in the field of chemical biology, in particular chemoselective ligation methods, renders this challenge possible. In this Minireview, we outline the different available synthetic strategies, some applications that already make use of this new generation of multivalent and multimodal architectures, and the challenges for future developments.*

1. Introduction

Since the late 1970s, dendrimers have evolved into a versatile new class of compounds that lies between small organic molecules and polymers. The stepwise synthesis of dendrimers affords highly branched and essentially monodisperse macromolecules with a well-defined number of peripheral groups.[1] While early research focused on the chemical synthesis and characterization of dendrimers, researchers soon began to investigate the properties and functions of dendrimers while exploring their applicability.[2] The tuneability of size, architecture, density, and surface groups render dendrimers a highly versatile platform for materials and biomedical research.[3] Dendrimers are used for a variety of applications including drug delivery, gene transfection, and molecular imaging.[4] The good control over the number of surface groups offers the opportunity to employ multivalent interactions to produce ligands with increased affinity and specificity for biological targets.[5] Dendrimers typically form spherical objects with diameters in the nanometer range, thus leading to a longer blood circulation time compared to small molecules. Furthermore, compared to normal polymers, the low polydispersity (close to 1.0) provides improved and reproducible pharmacokinetic behavior.[6] The biomedical applications of dendrimers are now at the forefront of research and many excellent reviews contain a wealth of information concerning most of the important dendrimers.[1b,c,2a,3a,4a–c,5a,7a,b]

Numerous research groups have reported the statistical modification of dendrimers comprising uniform surface groups; these dendrimers can be used in applications such as boron neutron capture therapy (BNCT),[8] magnetic resonance imaging (MRI),[9] or drug delivery.[10] However, statistical modification forfeits the advantage of monodispersity and the homogeneity of the material. With the rise of chemical biology, research topics such as multimodal imaging, targeted drug delivery, or combined targeted imaging and treatment evolved.[11] Those applications require the introduction of multiple functionalities in a highly controlled fashion, and have provided an impetus to take dendrimer research to the next stage. The development of a variety of chemoselective ligation reactions,[12] such as native chemical or Staudinger ligation,[12a] copper-assisted azide–alkyne cycloaddition (CuAAC),[12b,e] or the thiol–ene reaction,[12c] has helped overcome the synthetic challenge, although the extensive use of orthogonal protecting groups is usually still required. Nonetheless, methods that allow the controlled synthesis of well-defined dendritic materials that display at least three different functionalities (e.g., targeting, imaging, and therapy) will pave the way not only for a broad application in biomedical research but also for the transition from laboratories to clinics. In Section 2, we discuss selected

[*] Dr. L. Röglin, E. H. M. Lempens, Prof. Dr. E. W. Meijer
Laboratory of Chemical Biology
Department of Biomedical Engineering
Eindhoven University of Technology
PO Box 513, 5600 MB Eindhoven (The Netherlands)
Fax: (+31) 40-245-1036
E-mail: e.w.meijer@tue.nl

available synthetic routes towards well-defined multimodal (i.e., containing different functionalities) dendritic compounds, and focus on possible biomedical applications. In Section 3, we will discuss biomedical applications to which such materials have already been applied. We have selected examples, which, in our view, are the most illustrative examples of the last 10 years, to demonstrate the synthetic "tour de force" of well-defined multivalent and multimodal dendritic structures without being exhaustive. We will end with a short survey of future developments.

Lars Röglin studied chemistry at the University of Dortmund and received his Diploma in 2002. After finishing his Ph.D. studies in the field of bioorganic chemistry in the group of Prof. Dr. O. Seitz at the Humboldt-Universität zu Berlin, he joined the research group of Prof. Dr. E. W. Meijer for postdoctoral research on multivalent architectures for biomedical applications. He is currently working on targeting protein–protein interactions with small molecules at the Chemical Genomics Centre in Dortmund.

Edith Lempens received her M.Sc. degree in biomedical engineering in 2006 from the Eindhoven University of Technology. She spent a few month at The Scripps Research Institute La Jolla working with Dr. P. E. Dawson and is currently completing her Ph.D. in the group of Prof. Meijer. Her main research interest focuses on multivalent binding events in chemical biology.

Bert Meijer studied at the University of Groningen, where he received his Ph.D. in the field of organic chemistry in 1982. After a 10 year career in industry (Philips and DSM) he became head of the group Molecular Science & Technology at the Eindhoven University of Technology. His research is focused on the design, synthesis, characterization, and application of supramolecular architectures, with special emphasis on chirality, dendrimers, π-conjugated polymers, hydrogen-bonding architectures, and the noncovalent synthesis of complex molecular systems.

2. Strategies for the Synthesis of Well-Defined Multifunctional Materials

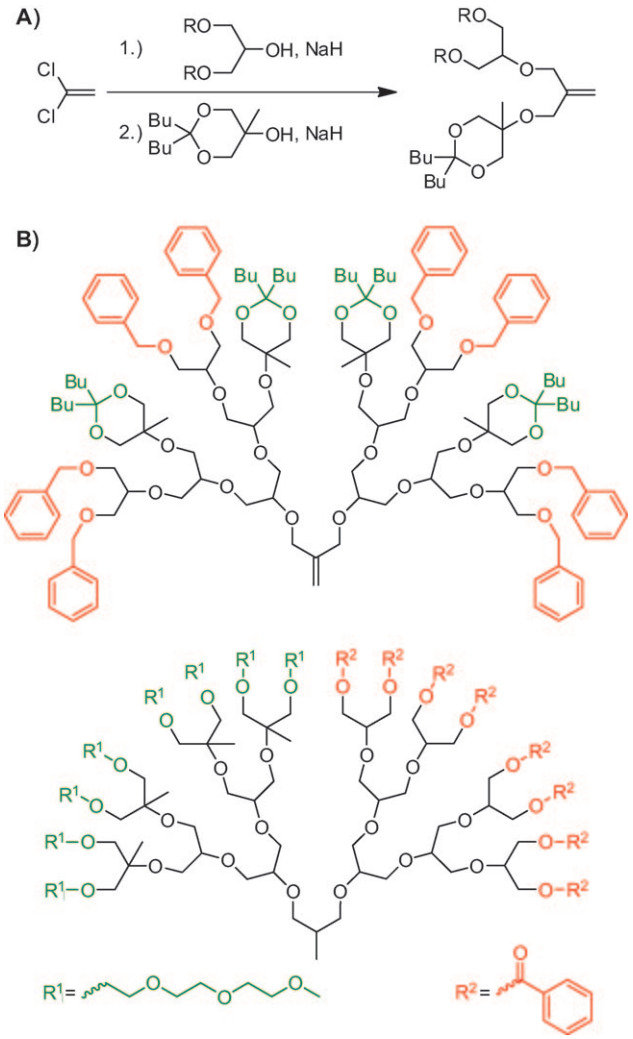
The key to the successful synthesis of well-defined, monodisperse dendritic structures is the accurate control over surface functionalities that can be addressed separately. This synthesis can be achieved, for example, by using orthogonally protected functional groups, chemoselectively addressable functionalities, or a combination of both options. Historically, dendrimers have been synthesized by using either convergent or divergent approaches, both of which have advantages and disadvantages. The divergent approach was introduced first by the research groups of Tomalia and Newkome, and is mostly used because of its ease in synthesis.[13] These dendrimers however often lack—certainly at higher generations—full control over purity and hence the exact number of end groups. Multifunctional materials are typically statistically or at best alternating at the periphery. The convergent approach was introduced by Hawker and Fréchet, and is synthetically more demanding.[14] However, this synthetic approach typically produces dendrimers of very high purity and allows the production of dendrons, which are useful either by themselves or as building blocks for asymmetric dendrimers with multifunctionalities at precise positions.

We first discuss examples of convergent or divergent/convergent strategies, all of which involve some steps that link branched units to a core. This approach is the more common one, with the exception of divergent solid-phase synthesis of peptide dendrimers. These dendrimers are widely used as so-called "multiple antigenic peptides", and the synthetic methodology for these compounds has been reviewed extensively elsewhere.[15] However, in recent years, peptide dendrimers have been combined with chemoselective ligation or conjugation methods. These approaches allow the controlled synthesis of well-defined multimodal macromolecules and are therefore also included. Finally, we discuss some examples of complete divergent processes that typically start from an orthogonally protected core molecule, with the preservation of orthogonality throughout the synthesis.

2.1. Divergent/convergent or Convergent Approaches

An early synthesis of dendrons that bear two different and orthogonal protecting groups was reported by Grayson and Fréchet,[16] who used a convergent strategy[14] for the synthesis of aliphatic polyether dendrons to gain precise control over the dendritic architecture. The key building block is 3-chloro-2-chloromethyl-1-propene (methallyl dichloride, MDC), which has two important properties. Firstly, the dual allylic chloride functionalities can be substituted in a Williamson-type reaction and secondly, the remaining alkene function can be transformed by hydroboration–oxidation to provide the hydroxy function required for the subsequent cycle. By using this strategy, it is possible to generate either dendrons in which the different protecting groups or functionalities are equally distributed (Scheme 1B, top) or dendrons in which the different functional groups are located on one half of the dendron (Scheme 1B, bottom). Lindhorst and co-workers subsequently used this strategy to synthesize polyether glycodendrons with galactose and mannose units (Scheme 2).[17] Dendrons that bear these biologically relevant sugar epitopes may serve as useful oligosaccharide mimetics in biological applications.

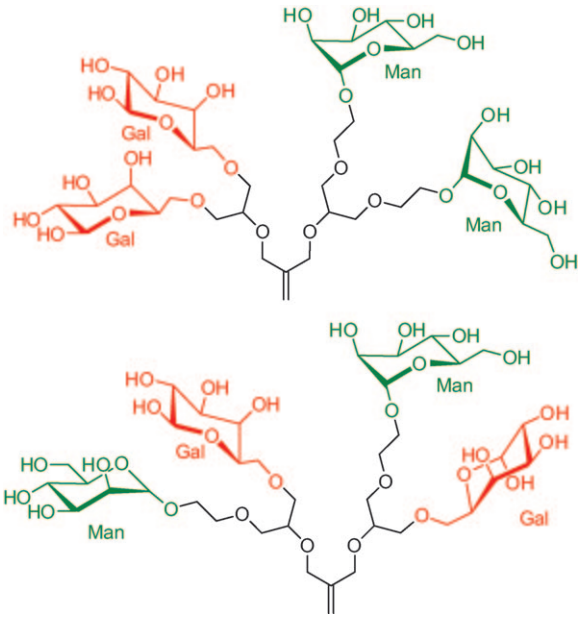
Another promising example for the potential of convergent approaches are triazene-based dendrimers reported by Steffensen and Simanek. By making use of the well-known control in triazine chemistry, a proof-of-principle experiment showed that this type of dendrimer can be synthesized to bear



Scheme 1. A) Desymmetrization of 3-chloro-2-chloromethyl-1-propene (after hydroboration–oxidation of the double bond, the building block can be used to synthesize the next generation); B) orthogonally functionalized dendrons synthesized by Grayson and Fréchet using the reaction shown in (A).[16]

either five or six different groups that can undergo further modification (Scheme 3).[18] These groups include unprotected as well as protected hydroxy groups, *tert*-butoxycarbonyl (Boc)-protected amines, and thiopyridyldisulfides. Thus, all types of conjugation reactions using hydroxy, amine, or thiol groups can be used to introduce the desired functionalities. Furthermore, five or six selectively addressable functionalities are more than sufficient for any practical application.

A more recent example focused on the use of an orthogonally protected Boc/Dde (1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl) variant, which allows easier synthesis and purification because of its higher stability compared to other groups such as the thiopyridyl moiety. This strategy allowed the synthesis of a dendrimer bearing 24 Boc- and 12 Dde-protected amines. To demonstrate the introduction of functional modifications, the Dde protecting group was first replaced using thiopyridyl-protected 3-thiolpropanoic acid *N*-hydroxysuccinimide ester and subsequently the

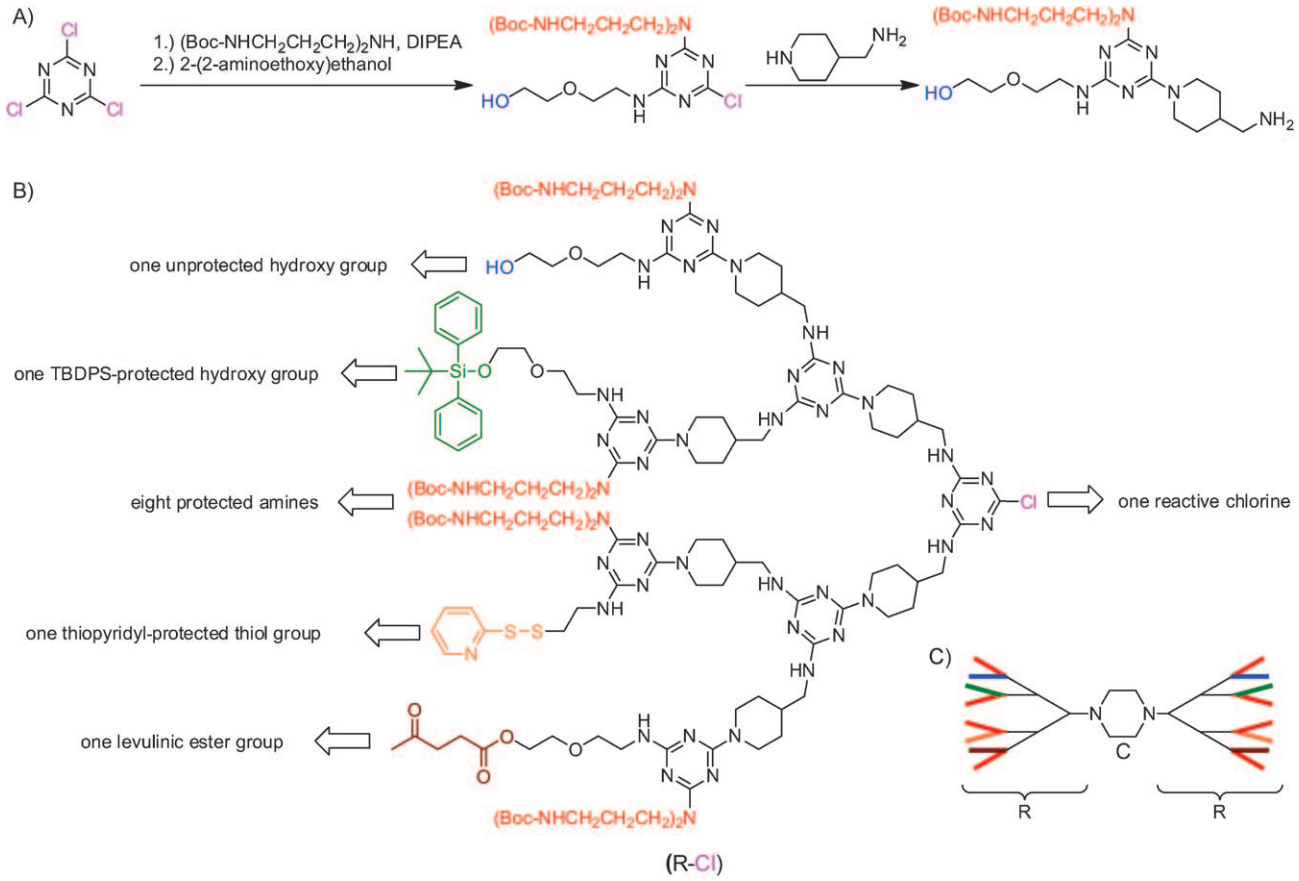


Scheme 2. Sugar-modified polyether dendrons synthesized by Lindhorst and co-workers using the synthetic strategy shown in Scheme 1.[17]

Boc-protected amines were PEGylated (PEG = polyethylene glycol) to result in a fully functionalized 57 kDa dendrimer.[19]

Weck and co-workers used monofunctionalized building blocks (Scheme 4A) to synthesize Newkome-type dendrimers that bear either one or two selectively addressable groups.[20] To achieve monofunctionalization, a half-dendrimer that bears one orthogonally protected hydroxy group was linked to another half-dendrimer comprising only *t*Bu esters (Scheme 4B). The combination of two half-dendrimers that bear a single selectively addressable group (one free and one acetylated hydroxy group) allows the synthesis of dendrimers that bear one aldehyde group suitable for further reactions such as the oxime ligation,[21] and one azide group suitable for CuAAC reactions (Scheme 4C). The final product features three different addressable moieties and thus should allow the attachment of all the key functional units for practical applications (targeting unit, drug, diagnostic label).

A special case of the convergent approach is the linkage of two dendritic wedges by using special connecting units or functionalities as key features. These connecting units can generate either a covalent or a noncovalent link between the two fragments. The CuAAC reaction was among the first to be used for this approach. Lee et al. screened different reaction conditions for the optimal cross-linking of Fréchet-type benzyl propargyl ether dendrons (Scheme 5A) or poly(amidoamine) (PAMAM) dendrons (Scheme 5B).[22] However, the conditions used for the conjugation of the Fréchet-type dendrons (toluene, 50 °C, >20 hours)[22a] may not be suitable for functionalized dendrons. On the other hand, the optimized conditions used for the PAMAM dendron (THF/water 4:1, room temperature, 4 hours)[22b] should allow linkage even with dendrons that bear rather sensitive functionalities.



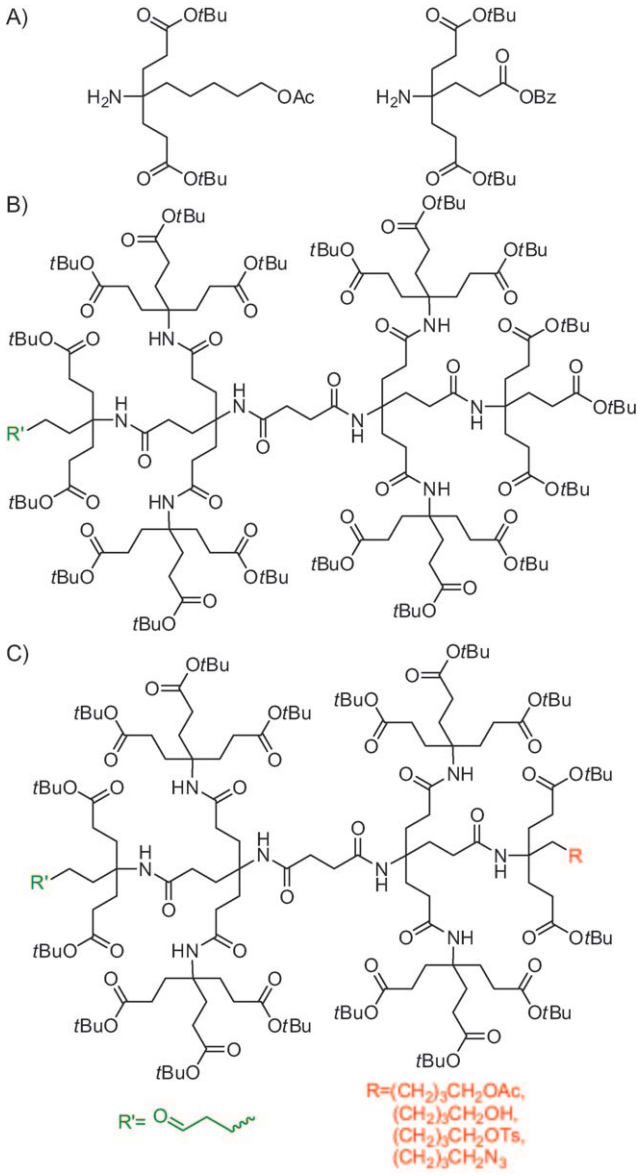
Scheme 3. A) Synthesis of the key intermediate by desymmetrization of cyanuric chloride; B) dendron that bears six selectively addressable functionalities; C) combination of two dendrons in (B) leads to a dendrimer that still bears five selectively addressable groups.[18]

The Diels–Alder cycloaddition is another selective and efficient conjugation reaction that has also been investigated for the conjugation of two half-dendrimers. Kose et al. used a furan-functionalized polyaryl ether dendron in combination with maleimide functionalized polyester dendrons as a model system (Scheme 6).[23] Yields for the model reaction were only given for reactions in benzene at 85 °C, which may be problematic in relation to functionalized dendrons. Full *exo*-stereoselectivity was only achieved with first-generation dendrimers. Since this selectivity affects only the core of the dendrimer, the effect on the overall structure of higher-generation dendrimers might be negligible. In addition, it has already been demonstrated that the reaction can be performed at room temperature, and can be used for peptides in aqueous systems.[24] Thus, this approach might also be applicable for peptide-functionalized dendrimers.

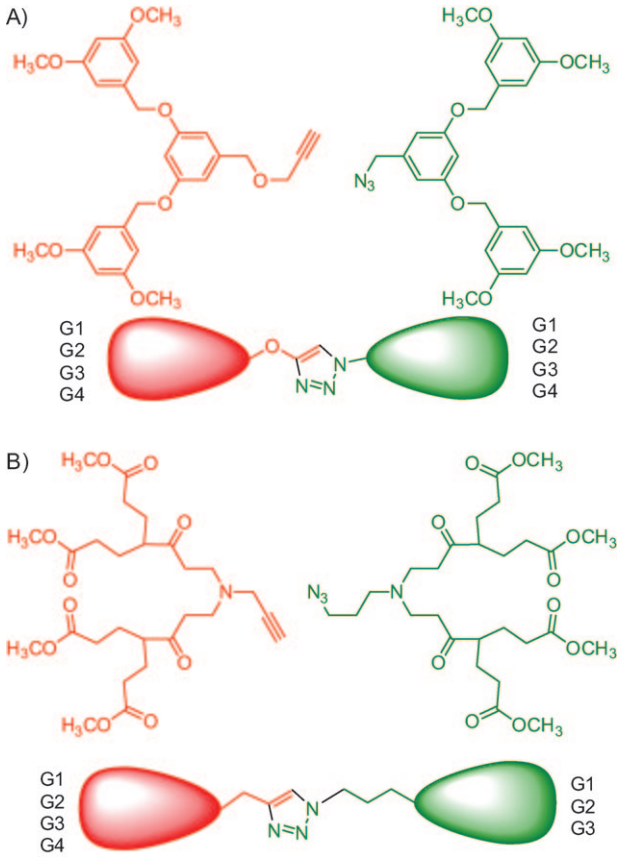
A conceptually different approach employs the noncovalent dimerization of two dendrons functionalized with ureidopyrimidine (Upy) units. This approach has been used to link oligoether or Newkome-type dendrons in a noncovalent fashion,[25] but is based on the hydrogen bonding between two Upy units and is thus limited to symmetric assemblies and apolar solvents such as $CHCl_3$. As a result, this concept seems to be unsuitable for applications in aqueous buffered in vitro or in vivo systems. A possible solution for this problem could be replacement of the Upy units with complementary DNA strands. Choi et al. have demonstrated that it is possible to use DNA strands to combine two asymetrically functionalized dendrimers.[26] In contrast to the Upy dimerization, DNA hybridization is suitable for application in aqueous systems and can also be used to design asymmetric systems. In fact, it is not even limited to the connection of just two elements, as demonstrated by Fréchet and co-workers for a three-component system.[27] Although the hybridization is only demonstrated with statistically modified dendrimers or unmodified dendrons in both cases, this approach undoubtedly also allows the linkage of well-defined, discrete functionalized dendrons.

2.2. Divergent Approaches

Divergent approaches for the synthesis of multifunctional dendrimers rely heavily on orthogonally protected building blocks. The most prominent example of this reaction type are peptide dendrimers, which have been reviewed extensively.[15] However, it should be noted that the use of peptide scaffolds has gained versatility in recent years, since it is no longer necessary to synthesize the whole construct sequentially because of the emergence of a number of different orthogonal chemoselective ligations methods.[12a,b,21a,28] Instead, the or-



Scheme 4. Dendrimers with one or two selectively addressable groups prepared by Weck and co-workers.[20]



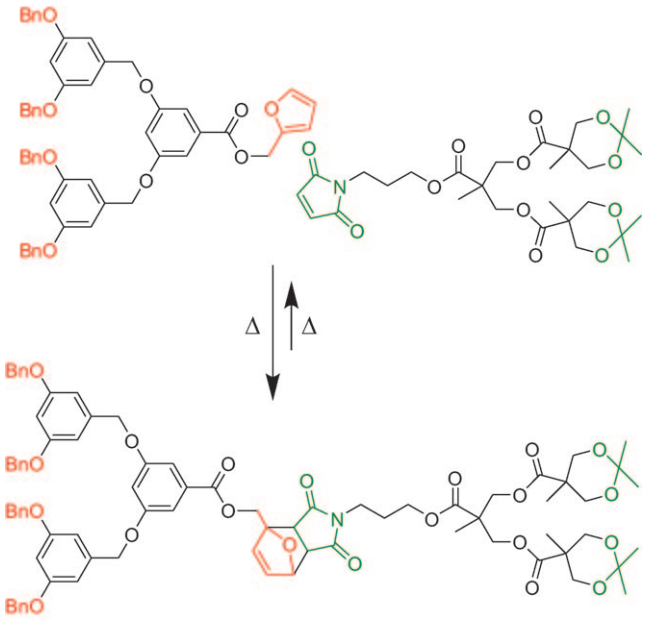
Scheme 5. Conjugation of two half dendrons using the CuAAC reaction.[22]

thogonality of the different protecting groups can be used to introduce the required reactive group for subsequent ligation of the desired functionalities.

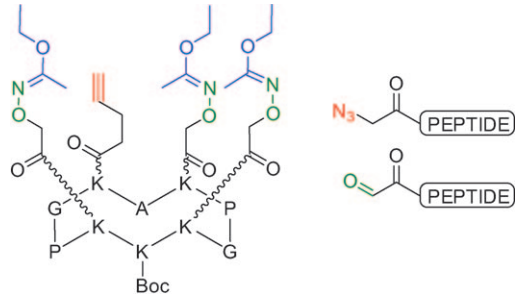
An interesting example of this approach has been recently reported by Galibert et al.,[29] who used the cyclopeptide scaffold introduced by Mutter et al. in 1988[30] as starting point for the introduction of two different peptide ligands by employing orthogonal chemoselective ligation methods (Scheme 7). This work shows that the combination of CuAAC and oxime chemistry allows addition of the two peptide ligands even in a one-pot reaction, thereby eliminating the necessity to purify intermediates and thus increasing the overall yield. However, conditions had to be found for the one-pot reaction that allow both a fast and stable oxime-bond formation and the CuAAC reaction, and rather unusual conditions of nanosized copper powder in a 5% trifluoroacetic acid (TFA) solution were applied. Furthermore, the underlying scaffold structure is limited to four or six addressable positions, as larger cyclopeptides are not expected to provide a conformationally defined core structure.

An early example of asymmetrical PAMAM dendrimers was reported by Martin and Twyman,[32] who used a mono-Boc-protected ethylene diamine unit as a starting point for their synthesis. After three rounds of standard PAMAM-synthesis, the terminal ester groups were replaced by isobutylamide units. Subsequent deprotection of the Boc units and three additional rounds of PAMAM synthesis eventually led to the asymmetrical G3 dendrimer that bears isobutylamide end groups on one half and methyl esters on the other.

Another approach has been developed by Fréchet and coworkers.[33] Instead of performing the entire synthesis in a divergent fashion, a strategy is presented in which the synthesized dendrimer is desymmetrized by using cyclic carbonates in the final steps. These cyclic carbonates were attached to different bis(hexamethylphosphoramide) (HMPA)-based dendrimers. The highly efficient and selective ring opening of a cyclic carbonate with an amine has been applied for polyurethane synthesis to result in a stable carbamate linkage. The conversion directly liberates an alcohol moiety that can be used for subsequent modifications, thus allowing the direct addition of the next functionality without requiring any functional-group interconversion or deprotection steps. However, one disadvantage of this



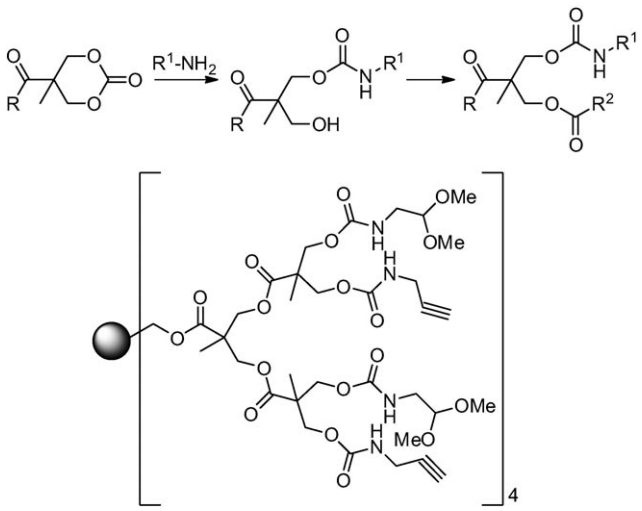
Scheme 6. Diels–Alder cycloaddition between two suitably functionalized dendrons.[23]



Scheme 7. Chemoselective ligations allow modification of a suitable functionalized cyclopeptide scaffold.[29] The 1-ethoxyethylidene (Eei, blue) protecting group was developed earlier by the same research group to prevent overacylation of the NH–O functionality.[31]

approach might be that the ratio between the two functionalities cannot be tuned, as equal numbers both functionalities are always present.

More recently, the potential of a related approach was investigated (Scheme 8).[34] Firstly, a trifunctional amino acid was coupled to a common symmetrical aliphatic ester dendritic core. The amines were subsequently PEGylated to leave the third amino acid function available for further conjugation, for example, radioiodination of tyrosine. However, it became apparent that quantitative functionalization of the PEGylated dendrimers was difficult, if not impossible, to achieve. In one example, the authors tried to attach the antitumor drug doxorubicin by a hydrazone bond to the side-chain carboxylic acid of an aspartate moiety. Since direct modification with a *tert*-butylcarbazate failed because of the lability of the α-amido ester bonds towards primary amines, the aspartate acid was first conjugated to a Boc-protected hydroxyhydrazide linker. The yield of this reaction was



Scheme 8. Final desymmetrization using cyclic carbonates.[34]

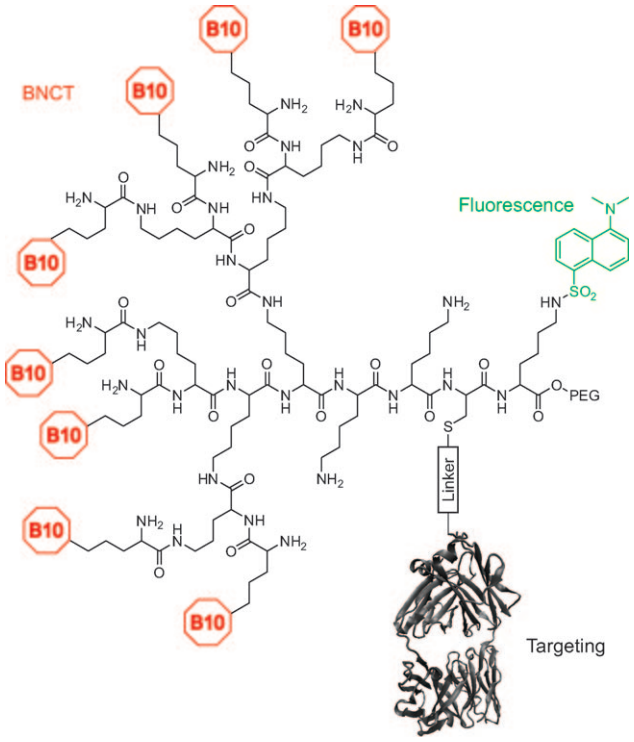
estimated to be roughly 50% by NMR studies. The same yield was obtained when the doxorubicin was conjugated to the liberated hydrazide. Thus, the overall doxorubicin loading was only 25%. Although this loading might be sufficient for the desired applications, it forfeits the advantage of dealing with exactly defined entities instead of statistically modified dendrimers.

3. Applications of Multivalent Multifunctional Materials

Functionalization of surface groups is required for the development of dendritic structures that can be used in biomedical applications. Homogeneous conjugation of pharmaceutical agents, targeting groups, and (radio)labels onto a single scaffold is a synthetic challenge, but is essential to arrive at single molecules for which the exact structures can be assigned. In this section, we discuss promising medical applications of multifunctional dendrimers including multivalent targeting, multimodal imaging, or combined imaging and drug delivery. The macromolecules presented can be categorized into dendritic wedges, merged dendritic wedges, or miscellaneous dendritic structures.

3.1. Dendritic Wedges

In 1996, Qualmann et al. were the first to report the synthesis of a defined multifunctional dendron based on a poly(lysine) core.[35] Simultaneous incorporation of eight carborane clusters at the outer sphere and an antibody fragment together with a fluorescent probe at the focal point revealed promising perspectives for its use in boron neutron capture therapy (Scheme 9). By using TentaGel PAP resin for the synthesis, an additional PEG tail was incorporated to increase the solubility of the probe in water. This example shows how four different functionalities can be conjugated in a highly controlled manner through fast and efficient solid-



Scheme 9. Schematic representation of a second-generation poly(lysine) dendritic wedge functionalized with eight carborane clusters (red), an antibody fragment, and a fluorescent probe (green).[35]

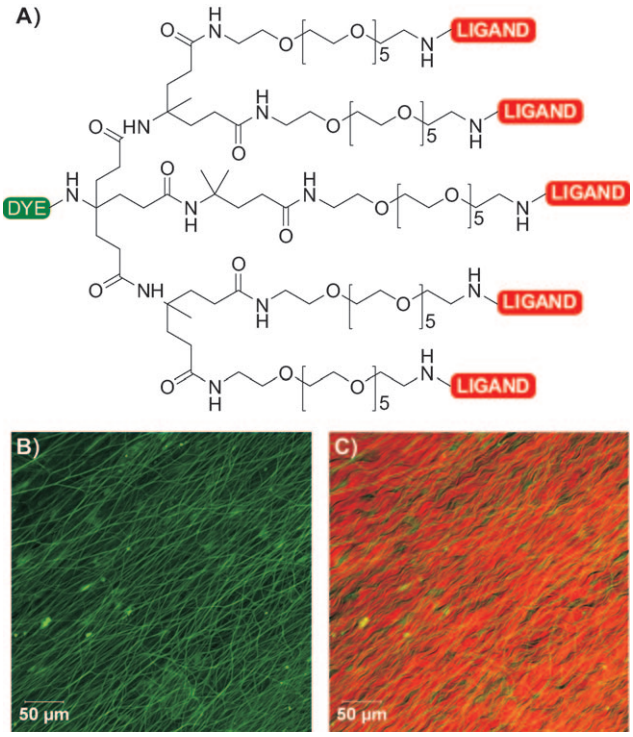
phase chemistry. Several years later, fourth-generation mannosylated poly(lysine) wedges with a fluorescent group at the C terminus were prepared by Kantchev et al. to mimic the mannose-receptor-mediated capture and uptake of pathogens by dendritic cells.[36] Conjugation of these glycodendrons is a potentially useful strategy to enhance the immunogenicity of, for example, peptides in vaccine design.[37] Since then, glycodendrimers have found widespread use. An overview of existing strategies and potential biological applications of glycodendrimers is given in the excellent review by Chabre and Roy.[38]

A strategy similar to that of Kantchev et al. was recently used by Lusvarghi et al. to assemble various generations of peptide-functionalized poly(lysine) wedges with a biotin group at the focal point. These wedges were studied for their potential use as biosensors in order to detect bacterial spores in air, water, or food supplies.[39] Direct comparison of the weakly binding monovalent spore-binding peptide with the divalent and tetravalent dendrons revealed an increase in affinity of one and two orders of magnitude, respectively.

Besides the use of poly(lysine) dendritic structures, several research groups have reported the functionalization of other types of dendrons. For example, PAMAM wedges have been condensed with DNA after modification of the focal point with a peptide-modified PEG. The peptide (WIFPWIQL) is capable of targeting the wedges to cells that express the 78 kDa glucose-regulated protein (GRP-78) identified in tumors. In vitro experiments showed that the dendron efficiently targeted and transfected prostate carcinoma cells and has the potential for clinical applications in tumor-targeted gene delivery.[40]

Kostiainen et al. also prepared precisely defined dendritic wedges that are biocompatible and show efficient gene transfection. First- and second-generation Newkome-type wedges modified with spermine at the periphery were used in this synthesis. Spermine, which is a naturally occurring polyamine, is capable of high-affinity binding to DNA as shown by an ethidium bromide displacement assay. Functionalization of the focal point with a maleimide group allowed attachment of proteins through their free thiol groups in the cysteine units. Conjugation of the adhesion protein hydrophobin (HFBI) to a second-generation spermine dendron generated a self-assembling amphiphile that promoted efficient gene transfection in vitro.[41]

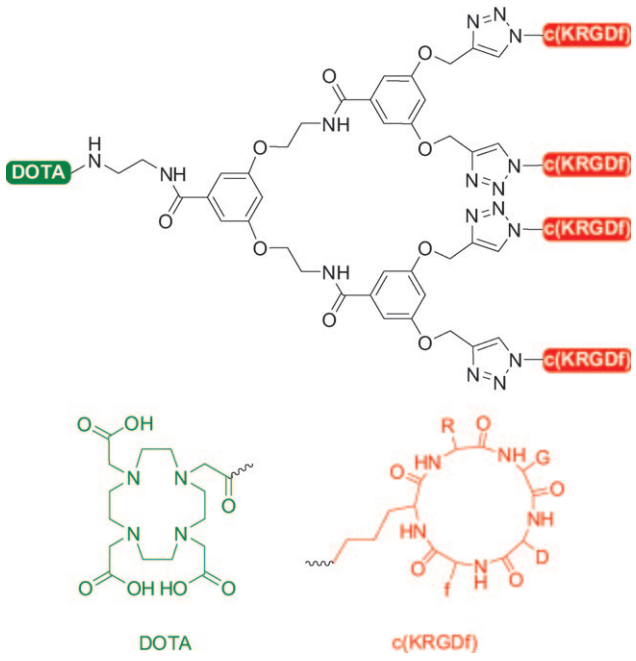
Another dendritic structure inspired by the Newkome-type dendrons is the labeled pentameric peptide wedge designed by our research group (Scheme 10A).[42] In this example, a versatile platform for the affinity enhancement of phage display derived peptides was created by mimicking key aspects of the multivalent architecture of the M13 phage head. The synthesis relied on an orthogonal protecting group strategy followed by a chemoselective ligation of the peptides. More specifically, PEG units were used to link acid-labile trityl-protected cysteine residues to the periphery, while an orthogonal base-labile fluorenylmethoxylcarbonyl (Fmoc) group at the focal point allowed incorporation of biotin or fluorescein labels. Collagen type I binding peptides (HVWMQAPGGG), which were identified by phage display,



Scheme 10. A) Pentavalent Newkome-type dendritic wedge mimicking the multivalent head of the M13-phage; B, C) laser scanning confocal microscopy images of porcine pericardium co-stained with fluorescein labeled dendron (green) and AlexaFluor568-labeled CNA35 (red).[42]

were coupled to the peripheral cysteine residues by native chemical ligation. Binding experiments on collagen type I revealed a 100-fold increase in the affinity of the phage mimic compared to the monovalent peptide. Incubation of pig pericardial tissue with the fluoresceine-labeled pentamer revealed a distinct fibrous network consisting of only collagen type I (Scheme 10B). Co-staining of the tissue with Alexa-Fluor568-labeled CNA35, which is a protein known to bind various types of collagen, demonstrated the specificity of the peptide-functionalized dendron (Scheme 10C). As a consequence, the dendron has the potential application as visualization probe for detecting diseases characterized by collagen turnover, for example, angiogenesis or atherosclerosis. Subsequently, a whole series of this type of dendritic structure with an arithmetic control over the degree of branching (AB_2, AB_3, AB_4, and AB_5) was reported.[43]

The use of multifunctional dendritic wedges for use in tumor targeting and tumor imaging was demonstrated by the synthesis of peptide dendrons with a DOTA label (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid). Monomeric, dimeric, and tetrameric amino acid dendrons were synthesized starting from 3-(hydroxymethyl)benzoate or 3,5-(dihydroxymethyl)benzoate and 2-bromoethylamine.[44] A microwave-assisted 1,3-dipolar cycloaddition was then performed to functionalize the dendritic alkynes with *N*-ε-azido-modified c(KRGDf) (Scheme 11). Selective binding of these cyclic peptides to αvβ3 integrins is thought to prevent tumor growth by antagonizing angiogenesis. After connection of the DOTA moiety to the dendritic core, solid-phase binding assays and biodistribution studies were performed. The tetrameric construct showed enhanced affinity for αvβ3 integrins and significant higher tumor uptake in athymic mice with a subcutaneously growing SK-RC-52 renal cell carcinoma.[45]



Scheme 11. Structure of a DOTA-conjugated (green) tetravalent cyclo-(RGDfK) peptide (red) dendrimer.[45]

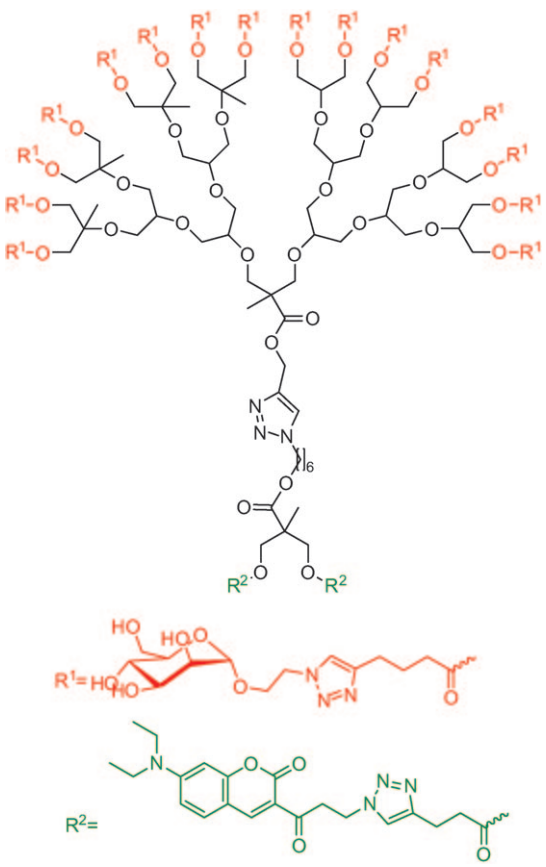
In another study, Wu et al. explored the use of synthetic glycodendrons as a new strategy to coat single-walled carbon nanotubes (SWNTs) and to promote the binding of the SWNTs to cells. These nanotubes have been employed for imaging, drug delivery, and cancer cell targeting, but modifications are required to diminish their cytotoxicity. Bifunctional dendrimers based on the biocompatible building block 2,2-bis(hydroxymethyl)propionic acid (G2 and G3) were functionalized with a variety of peripheral carbohydrate moieties. Conjugation of a pyrene group to the focal point allowed binding to SWNTs through π–π interactions.[46] In vitro experiments with these biocompatible carbon nanotubes revealed receptor specific binding and labeling of the cell membrane.

3.2. Merged Dendritic Wedges

Only dendritic wedges with a single modification at the focal point have been discussed so far. However, several research groups have reported the preparation of functional dendrimers by merging two dendritic blocks together. Wu et al. used the 2,2-bis(hydroxymethyl)propionic acid building block for the synthesis of dendrons with either an alkyne or an azide functionality at the focal point. Two series were synthesized up to the fourth generation, and coupling of various blocks proceeded smoothly using copper(I)-catalyzed azide–alkyne cycloaddition. Finally, an asymmetrical dendrimer with two 7-diethylaminocoumarin dyes and sixteen mannose groups (Scheme 12) was prepared, and exhibited a potency that was 240 times greater than monomeric mannose in the hemagglutination assay.[47]

A similar concept was used for the development of multivalent target-specific MRI contrast agents for potential in vivo imaging of cardiovascular diseases. Two poly(lysine) wedges, one of which has a thioester at its focal point and the other a cysteine residue, were coupled by native chemical ligation. The chemical structure of the dendrimer that consists of a second-generation DTPA wedge and a first-generation peptide wedge (GRGDS) is shown in Scheme 13.[48]

Deguise et al. used the azide–alkyne cycloaddition for the synthesis of well-defined glycodendrimers containing fucoside residues on one side and galactoside residues on the other. These two carbohydrates inhibit either PA-IL or PA-IIL lectins present in gram-negative bacteria such as *Pseudomonas aeruginosa* and are therefore interesting therapeutic agents for the prevention of bacterial infections. The repeating unit for the dendron synthesis was an aromatic diazido acid, and merging of the two building blocks was based on the formation of amide linkages with a bisamine function.[49] Hetero-bifunctional dendrimers with up to eight fucoside and eight galactoside residues showed binding and clustering of both PA-IL and PA-IIL lectins. The three examples discussed above show that the ratio between the number of labels and the number of targeting units can be controlled and optimized by merging two dendrons. Furthermore, both functionalities are physically separated from each other in distinct clusters, thus allowing optimal presentation of ligands for binding to receptors.



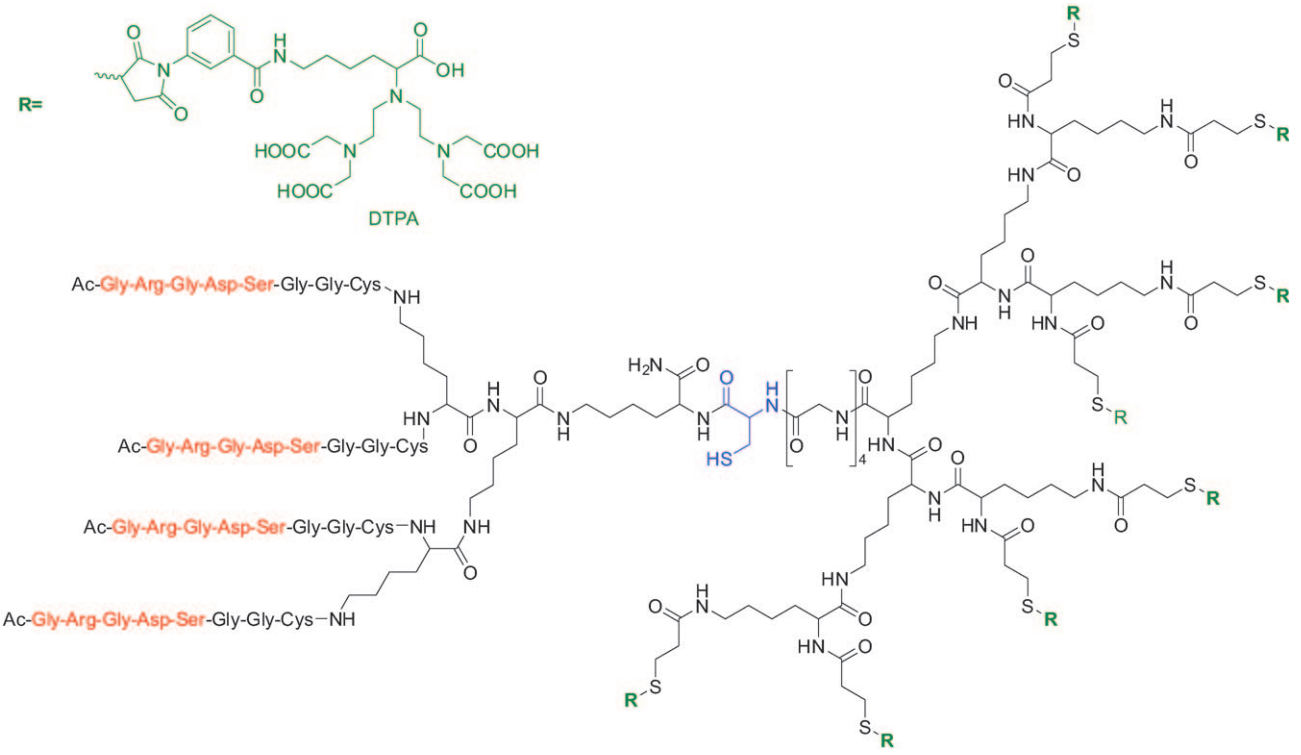
Scheme 12. Asymmetric dendrimer containing sixteen mannose groups and two coumarin fluorophores.[47]

3.3. Miscellaneous Dendritic Structures

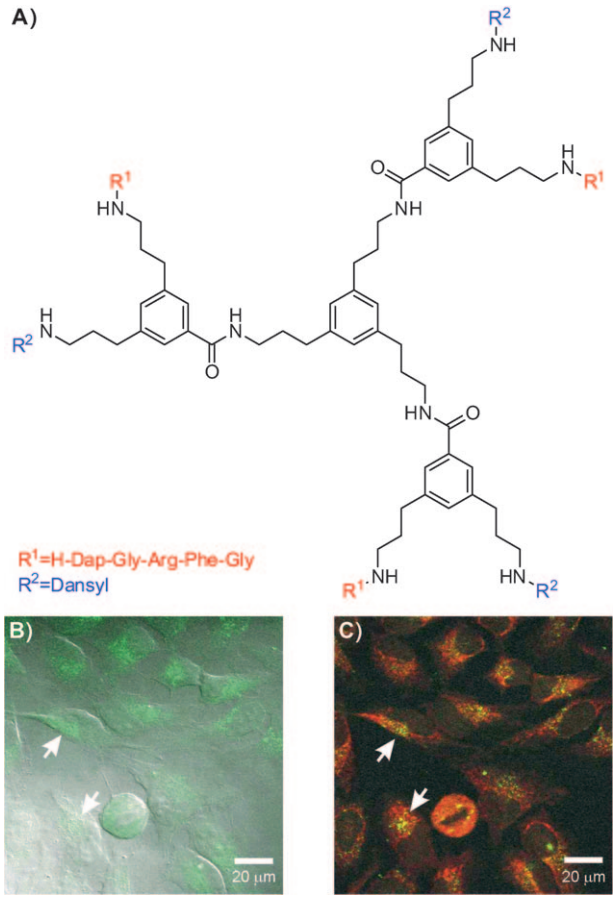
Besides the use of dendritic wedges for the synthesis of multifunctional dendrimers, a few other approaches have been explored. Fuchs et al. prepared a first-generation bifunctional polyamidoamine dendrimer by using the orthogonally protected 3,5-bis(3-aminopropyl)benzoic acid as a branching unit (Scheme 14 A).[50] Three fluorescent dansyl tags for intracellular detection and three enzymatically cleavable oligopeptides (GRFG) were incorporated into the dendrimer. Furthermore a 2,3-D,L-diaminopropionic acid (Dap) ligand was introduced to the N terminus of the peptide for potential complexation of anticancer-active Pt^{2+} ions. Confocal fluorescence microscopy was used to confirm the rapid (within 10 minutes) internalization of these dendrimers in HeLa cells. After more than 18 hours, the dendrimers appeared in clusters near the cell nucleus, thus indicating enrichment at the Golgi apparatus (Scheme 14 B). Coupling of antitumor agents to the cleavable peptides might generate promising structures for targeted drug delivery.[51]

Another drug delivery system based on PEGylated triazine dendrimers derivatized with two groups for radioiodination and 16 anticancer agents, was investigated by Lim and Simanek. The synthetic route is based on stepwise reactions of cyanuric chloride with amine nucleophiles, and gives rise to an interesting dendritic structure with three different, precisely defined functional groups.[52]

From the examples presented herein, it is clear that the unique well-defined architecture of dendritic structures is ideally suited for use in biomedical applications. Most of the



Scheme 13. Combination of two lysine dendritic wedges by native chemical ligation for targeted MRI.[48]



Scheme 14. A) First-generation multifunctional dendrimer that bears a pentapeptide with a cathepsin B cleavage site, chelating ligands for complexation with Pt^{2+} ions, and a dansyl fluorophore; B, C) microscopy images of Hela cells incubated with the dendrimer (green) for 18 h. The inner nuclear membrane and parts of the endoplasmatic reticulum are stained with antibodies against LUMA (red). Arrows indicate clusters of labeled dendrimers.[51]

reported structures are based on dendritic wedges that bear multiple ligands at the periphery and a single label (fluorescent dye or contrast agent) at the focal point. However, it is more favorable to also have multiple labels for visualization during in vivo measurements. This issue is addressed in the few examples of merged dendritic wedges that currently exist where the ratio between number of labels and number of targeting units can be controlled. Further interdisciplinary research between synthetic organic chemists and chemical biologists will be needed to fully benefit from the advantages of well-defined multivalent and multifunctional macromolecules.

4. Conclusion

In recent years, the benefits of the controlled synthesis of monodisperse, multivalent, and multimodal materials have been recognized. A new generation of dendritic structures for biomedical applications has been synthesized by using orthogonal protecting group strategies, which, in many cases, are combined with chemoselective ligation methods. From the reported applications, it has become evident that the defined introduction of at least three different functionalities (e.g., targeting, imaging, and treatment) is a synthetic challenge, but is essential for future biomedical materials. For example, the multifunctional dendron prepared by Steffensen and Simanek (Scheme 3A) requires a multistep total synthesis consisting of no less than 10 convergent reactions. Fundamental questions regarding the overall size of the structures, number of functional groups required, and spacer lengths are still largely unexplored. However, the molecules need to be analyzed in order to fine-tune structure–function relationships and to make the translation from the laboratory to the clinic. Arrival at the ultimate structure for targeting, imaging, and/or delivery requires the best possible synergy between disciplines with synthesis as a key role. It is now realized that the breakthroughs in synthesis as often applied to the preparation of drugs have to be employed in the growing field of chemical biology. The synthesis of multivalent and multimodal architectures will gain more interest and importance in the years to come.

L.R. and E.H.M.L. contributed equally to this work. L.R. is grateful for an NWO Rubicon fellowship. This work was supported by a grant from NWO (VIDI 700 56.428). We gratefully acknowledge Dr. M. Merkx for critically reviewing the manuscript.

Received: June 30, 2010
Published online: November 29, 2010

[1] a) D. A. Tomalia, A. M. Naylor, W. A. Goddard, *Angew. Chem.* **1990**, *102*, 119; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 138; b) S. M. Grayson, J. M. J. Fréchet, *Chem. Rev.* **2001**, *101*, 3819; c) G. R. Newkome, C. D. Shreiner, *Polymer* **2008**, *49*, 1.
[2] a) A. M. Caminade, J. P. Majoral, *Acc. Chem. Res.* **2004**, *37*, 341; b) D. Astruc, E. Boisselier, C. Ornelas, *Chem. Rev.* **2010**, *110*, 1857.
[3] a) C. C. Lee, J. A. MacKay, J. M. J. Fréchet, F. C. Szoka, *Nat. Biotechnol.* **2005**, *23*, 1517; b) H. Rami, K. Ashok, *Macromol. Rapid Commun.* **2010**, *31*, 947.
[4] a) S.-E. Stiriba, H. Frey, R. Haag, *Angew. Chem.* **2002**, *114*, 1385; *Angew. Chem. Int. Ed.* **2002**, *41*, 1329; b) E. R. Gillies, J. M. J. Fréchet, *Drug Discovery Today* **2005**, *10*, 35; c) S. Svenson, D. A. Tomalia, *Adv. Drug Delivery Rev.* **2005**, *57*, 2106; d) M. Guillot-Nieckowski, S. Eisler, F. Diederich, *New J. Chem.* **2007**, *31*, 1111; e) J. B. Wolinsky, M. W. Grinstaff, *Adv. Drug Delivery Rev.* **2008**, *60*, 1037.
[5] a) R. Haag, F. Kratz, *Angew. Chem.* **2006**, *118*, 1218; *Angew. Chem. Int. Ed.* **2006**, *45*, 1198; b) L. L. Kiessling, J. E. Gestwicki, L. E. Strong, *Angew. Chem.* **2006**, *118*, 2408; *Angew. Chem. Int. Ed.* **2006**, *45*, 2348; c) C. A. Hunter, H. L. Anderson, *Angew. Chem.* **2009**, *121*, 7624; *Angew. Chem. Int. Ed.* **2009**, *48*, 7488.
[6] R. Duncan, L. Izzo, *Adv. Drug Delivery Rev.* **2005**, *57*, 2215.
[7] a) R. Esfand, D. A. Tomalia, *Drug Discovery Today* **2001**, *6*, 427; b) B. Helms, E. W. Meijer, *Science* **2006**, *313*, 929.
[8] a) W. Yang, R. F. Barth, D. M. Adams, M. J. Ciesielski, R. A. Fenstermaker, S. Shukla, W. Tjarks, M. A. Caligiuri, *Cancer Res.* **2002**, *62*, 6552; b) S. Shukla, G. Wu, M. Chatterjee, W. Yang, M. Sekido, L. A. Diop, R. Mueller, J. J. Sudimack, R. J. Lee, R. F. Barth, W. Tjarks, *Bioconjugate Chem.* **2003**, *14*, 158; c) G. Wu, R. F. Barth, W. Yang, M. Chatterjee, W. Tjarks, M. J. Ciesielski,

R. A. Fenstermaker, *Bioconjugate Chem.* **2004**, *15*, 185; d) W. Yang, R. F. Barth, G. Wu, A. K. Bandyopadhyaya, B. T. S. Thirumamagal, W. Tjarks, P. J. Binns, K. Riley, H. Patel, J. A. Coderre, M. J. Ciesielski, R. A. Fenstermaker, *Appl. Radiat. Isot.* **2004**, *61*, 981; e) W. Yang, R. F. Barth, G. Wu, S. Kawabata, T. J. Sferra, A. K. Bandyopadhyaya, W. Tjarks, A. K. Ferketich, M. L. Moeschberger, P. J. Binns, K. J. Riley, J. A. Coderre, M. J. Ciesielski, R. A. Fenstermaker, C. J. Wikstrand, *Clin. Cancer Res.* **2006**, *12*, 3792.

[9] a) V. S. Talanov, C. A. S. Regino, H. Kobayashi, M. Bernardo, P. L. Choyke, M. W. Brechbiel, *Nano Lett.* **2006**, *6*, 1459; b) Y. Koyama, V. S. Talanov, M. Bernardo, Y. Hama, C. A. S. Regino, M. W. Brechbiel, P. L. Choyke, H. Kobayashi, *J. Magn. Reson. Imaging* **2007**, *25*, 866; c) S. Langereis, A. Dirksen, T. M. Hackeng, M. H. P. van Genderen, E. W. Meijer, *New J. Chem.* **2007**, *31*, 1152; d) C. A. Boswell, P. K. Eck, C. A. S. Regino, M. Bernardo, K. J. Wong, D. E. Milenic, P. L. Choyke, M. W. Brechbiel, *Mol. Pharm.* **2008**, *5*, 527; e) S. D. Swanson, J. F. Kukowska-Latallo, A. K. Patri, C. Y. Chen, S. Ge, Z. Y. Cao, A. Kotlyar, A. T. East, J. R. Baker, *Int. J. Nanomed.* **2008**, *3*, 201.

[10] G. Spataro, F. Malecaze, C. O. Turrin, V. Soler, C. Duhayon, P. P. Elena, J. P. Majoral, A. M. Caminade, *Eur. J. Med. Chem.* **2010**, *45*, 326.

[11] A. Louie, *Chem. Rev.* **2010**, *110*, 3146.

[12] a) M. Köhn, R. Breinbauer, *Angew. Chem.* **2004**, *116*, 3168; *Angew. Chem. Int. Ed.* **2004**, *43*, 3106; b) J. F. Lutz, *Angew. Chem.* **2007**, *119*, 1036; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018; c) A. Dondoni, *Angew. Chem.* **2008**, *120*, 9133; *Angew. Chem. Int. Ed.* **2008**, *47*, 8995; d) C. P. R. Hackenberger, D. Schwarzer, *Angew. Chem.* **2008**, *120*, 10182; *Angew. Chem. Int. Ed.* **2008**, *47*, 10030; e) J. F. Lutz, H. G. Börner, *Prog. Polym. Sci.* **2008**, *33*, 1.

[13] a) G. R. Newkome, Z. Q. Yao, G. R. Baker, V. K. Gupta, *J. Org. Chem.* **1985**, *50*, 2003; b) D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, P. Smith, *Polym. J.* **1985**, *17*, 117.

[14] C. J. Hawker, J. M. J. Fréchet, *J. Am. Chem. Soc.* **1990**, *112*, 7638.

[15] a) L. Crespo, G. Sanclimens, M. Pons, E. Giralt, M. Royo, F. Albericio, *Chem. Rev.* **2005**, *105*, 1663; b) P. Niederhafner, J. Sebestik, J. Jezek, *J. Pept. Sci.* **2005**, *11*, 757; c) T. Darbre, J.-L. Reymond, *Acc. Chem. Res.* **2006**, *39*, 925; d) P. Niederhafner, M. Reinis, J. Sebestik, J. Jezek, *J. Pept. Sci.* **2008**, *14*, 556; e) P. Niederhafner, J. Sebestik, J. Jezek, *J. Pept. Sci.* **2008**, *14*, 2; f) P. Niederhafner, J. Sebestik, J. Jezek, *J. Pept. Sci.* **2008**, *14*, 44.

[16] S. M. Grayson, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2000**, *122*, 10335.

[17] K. Elsner, M. M. K. Boysen, T. K. Lindhorst, *Carbohydr. Res.* **2007**, *342*, 1715.

[18] M. B. Steffensen, E. E. Simanek, *Angew. Chem.* **2004**, *116*, 5290; *Angew. Chem. Int. Ed.* **2004**, *43*, 5178.

[19] A. P. Umali, H. L. Crampton, E. E. Simanek, *J. Org. Chem.* **2007**, *72*, 9866.

[20] P. Goyal, K. Yoon, M. Weck, *Chem. Eur. J.* **2007**, *13*, 8801.

[21] a) O. Melnyk, J. A. Fehrentz, J. Martinez, H. Gras-Masse, *Biopolymers* **2000**, *55*, 165; b) M. A. Gauthier, H. A. Klok, *Chem. Commun.* **2008**, 2591.

[22] a) J. W. Lee, B. K. Kim, *Synthesis* **2006**, 615; b) J. W. Lee, B. K. Kim, H. J. Kim, S. C. Han, W. S. Shin, S. H. Jin, *Macromolecules* **2006**, *39*, 2418; c) J. W. Lee, J. H. Kim, B. K. Kim, W. S. Shin, S. H. Jin, *Tetrahedron* **2006**, *62*, 894; d) J. W. Lee, J. H. Kim, H. J. Kim, S. C. Han, J. H. Kim, W. S. Shin, S.-H. Jin, *Bioconjugate Chem.* **2007**, *18*, 579.

[23] M. M. Kose, G. Yesilbag, A. Sanyal, *Org. Lett.* **2008**, *10*, 2353.

[24] a) A. D. de Araújo, J. M. Palomo, J. Cramer, M. Köhn, H. Schröder, R. Wacker, C. Niemeyer, K. Alexandrov, H. Waldmann, *Angew. Chem.* **2006**, *118*, 302; *Angew. Chem. Int. Ed.* **2006**, *45*, 296; b) A. D. de Araújo, J. M. Palomo, J. Cramer, O. Seitz, K. Alexandrov, H. Waldmann, *Chem. Eur. J.* **2006**, *12*, 6095.

[25] a) H. Sun, A. E. Kaifer, *Org. Lett.* **2005**, *7*, 3845; b) C. H. Wong, H. F. Chow, S. K. Hui, K. H. Sze, *Org. Lett.* **2006**, *8*, 1811.

[26] Y. Choi, T. Thomas, A. Kotlyar, M. T. Islam, J. J. R. Baker, *Chem. Biol.* **2005**, *12*, 35.

[27] S. L. Goh, M. B. Francis, J. M. J. Fréchet, *Chem. Commun.* **2002**, 2954.

[28] C. R. Becer, R. Hoogenboom, U. S. Schubert, *Angew. Chem.* **2009**, *121*, 4998; *Angew. Chem. Int. Ed.* **2009**, *48*, 4900.

[29] M. Galibert, P. Dumy, D. Boturyn, *Angew. Chem.* **2009**, *121*, 2614; *Angew. Chem. Int. Ed.* **2009**, *48*, 2576.

[30] a) M. Mutter, E. Altmann, K. H. Altmann, R. Hersperger, P. Koziej, K. Nebel, G. Tuchscherer, S. Vuilleumier, H. U. Gremlich, K. Müller, *Helv. Chim. Acta* **1988**, *71*, 835; b) M. Mutter, G. Tuchscherer, *Makromol. Chem. Rapid Commun.* **1988**, *9*, 437.

[31] S. Foillard, M. O. Rasmussen, J. Razkin, D. Boturyn, P. Dumy, *J. Org. Chem.* **2008**, *73*, 983.

[32] I. K. Martin, L. J. Twyman, *Tetrahedron Lett.* **2001**, *42*, 1119.

[33] A. P. Goodwin, S. S. Lam, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2007**, *129*, 6994.

[34] S. J. Guillaudeu, M. E. Fox, Y. M. Haidar, E. E. Dy, F. C. Szoka, J. M. J. Fréchet, *Bioconjugate Chem.* **2008**, *19*, 461.

[35] B. Qualmann, M. M. Kessels, H.-J. Musiol, W. D. Sierralta, P. W. Jungblut, L. Moroder, *Angew. Chem.* **1996**, *108*, 970; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 909.

[36] E. A. B. Kantchev, C.-C. Chang, D.- K. Chang, *Biopolymers* **2006**, *84*, 232.

[37] E. A. B. Kantchev, C.-C. Chang, S.-F. Cheng, A.-C. Roche, D.-K. Chang, *Org. Biomol. Chem.* **2008**, *6*, 1377.

[38] Y. M. Chabre, R. Roy, *Curr. Top. Med. Chem.* **2008**, *8*, 1237.

[39] S. Lusvarghi, J. M. Kim, Y. Creeger, B. A. Armitage, *Org. Biomol. Chem.* **2009**, *7*, 1815.

[40] K. C. Wood, S. M. Azarin, W. Arap, R. Pasqualini, R. Langer, P. T. Hammond, *Bioconjugate Chem.* **2008**, *19*, 403.

[41] M. A. Kostiainen, G. R. Szilvay, J. Lehtinen, D. K. Smith, M. B. Linder, A. Urtti, O. Ikkala, *ACS Nano* **2007**, *1*, 103.

[42] B. A. Helms, S. W. A. Reulen, S. Nijhuis, P. T. H. M. de Graaf-Heuvelmans, M. Merkx, E. W. Meijer, *J. Am. Chem. Soc.* **2009**, *131*, 11683.

[43] E. H. M. Lempens, B. A. Helms, A. R. Bayles, M. Merkx, E. W. Meijer, *Eur. J. Org. Chem.* **2010**, 111.

[44] S. J. E. Mulders, A. J. Brouwer, P. G. J. van der Meer, R. M. J. Liskamp, *Tetrahedron Lett.* **1997**, *38*, 631.

[45] I. Dijkgraaf, A. Y. Rijnders, A. Soede, A. C. Dechesne, G. W. Van Esse, A. J. Brouwer, F. H. M. Corstens, O. C. Boerman, D. T. S. Rijkers, R. M. J. Liskamp, *Org. Biomol. Chem.* **2007**, *5*, 935.

[46] P. Wu, X. Chen, N. Hu, U. C. Tam, O. Blixt, A. Zettl, C. R. Bertozzi, *Angew. Chem.* **2008**, *120*, 5100; *Angew. Chem. Int. Ed.* **2008**, *47*, 5022.

[47] 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.

[48] A. Dirksen, E. W. Meijer, W. Adriaens, T. M. Hackeng, *Chem. Commun.* **2006**, 1667.

[49] I. Deguise, D. Lagnoux, R. Roy, *New J. Chem.* **2007**, *31*, 1321.

[50] S. Fuchs, T. Kapp, H. Otto, T. Schöneberg, P. Franke, R. Gust, A. D. Schlüter, *Chem. Eur. J.* **2004**, *10*, 1167.

[51] S. Fuchs, H. Otto, S. Jehle, P. Henklein, A. D. Schlüter, *Chem. Commun.* **2005**, 1830.

[52] J. Lim, E. E. Simanek, *Org. Lett.* **2008**, *10*, 201.

