Peptide and Amide Bond-Containing Dendrimers

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Received March 8, 2004

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1. Introduction

1.1. Brief History of Dendrimers

Dendrimers are macromolecules comprised of a series of branches extending outward from an inner core.[1,2] The word dendrimer originates from the Greek *dendron*, meaning "tree". Ideally, they are perfectly monodisperse macromolecules with a regular and highly branched three-dimensional architecture. Dendrimers are usually produced in an iterative sequence of reaction steps, in which each additional iteration leads to a higher generation material. Dendrimers possess three distinct parts, each of which can be modulated: (i) a core, (ii) branching units, and (iii) branches (Figure 1).

As discussed by Newkome et al.,[1] progress toward the deliberate construction of macromolecules possessing branched architecture can be considered to have occurred in three periods. The first period occurred from the late 1860s to the early 1940s, when branched structures were considered responsible for the insolubility of, and intractable materials formed in, polymerization reactions. Synthetic control, mechanical separations, and physical characterization were primitive at that time, and isolation and proof of structure were simply not feasible.

The early 1940s to the late 1970s marked the second period, in which branched structures were considered primarily from a theoretical perspective. Initial attempts at their preparation employed classical or single-pot polymerization of functionally differentiated monomers.

The late 1970s and early 1980s, considered as the start of the third period of development, yielded preliminary success toward macromolecular assembly based on the iterative method that would become the cornerstone of dendrimeric chemistry. During this period, control of macroassembly construction was optimized. Advances in physical isolation and purification, as well as the introduction of diverse spectroscopic procedures for characterization, helped to provide the level of sophistication necessary for supporting the emerging field. Thus, Vögtle reported the first example of an iterative synthetic procedure applied toward well-defined branched structures,[3] naming it "cascade synthesis". A few years later, in the early 1980s, Denkewalter[4] patented the synthesis

10.1021/cr030449l CCC: $53.50
Published on Web 04/23/2005

Laia Crespo was born in Barcelona in 1974. After a first degree in Chemistry from the University of Barcelona, Spain, she received her Ph.D. from the same University in 2002. Her thesis work, under the supervision of Professors Fernando Albericio and Ernest Giralt, dealt with the synthesis and biophysical characterization of peptide dendrimers based on polyproline helices. She then joined Spirogen Ltd., London, U.K., as Research Scientist; there she developed combinatorial chemistry methods for the synthesis of DNA-binding peptides. Since 2004 she has been a member of the Combinatorial Chemistry team at Medivir U.K. Ltd. where her research involves the synthesis of polymerase and protease inhibitors.

Glòria Sanclimens was born in 1975. She received B.S. and Master's degrees in chemistry from the University of Barcelona in 1997 and 2000, respectively. She currently works in the Bioorganic Chemistry Department of the Spanish National Council of Scientific Research (CSIC) in Barcelona. Presently, Miss Sanclimens is preparing to defend her doctoral thesis on the synthesis of dendrimers at the University of Barcelona. She has coauthored 10 publications, including research papers and conference proceedings. Her current research interests encompass biopolymers, drug delivery, and drug discovery.

of L-lysine-based dendrimers up to the tenth generation.

1.2. Peptide Dendrimers

Peptide dendrimers can generally be defined as macromolecules that contain peptide bonds in their structure. Two categories of dendrimers, classified according to the role that amino acids play in their structure, are herein defined.

1.2.1. Types I and II: Covalent Peptide Dendrimers

These dendrimers contain either natural or nonnatural amino acids in their framework. Amino acids can be incorporated into the dendrimer core, building blocks, or branching units (Figure 2a), as part of peptide fragments used as building blocks (Figure 2b), or as peptides or amino acids grafted onto the surface of the organic core (Figure 2c).

Miquel Pons was born in 1956. After having earned B.S. degrees in Chemistry and Biology from the University of Barcelona, he received his Ph.D. in Biophysical Chemistry from the University of London in 1983. In 2003 he was promoted to Full Professor in the Department of Organic Chemistry at the University of Barcelona. Dr. Pons is a member of the Barcelona Biomedical Reserarch Institute and heads the laboratory of Biomolecular NMR at the Barcelona Science Park. His primary research interests are NMR and structural chemistry, specifically, NMR studies of protein–protein and protein–ligand interactions, as well as supramolecular chemistry. He is the author of more than 100 articles. Dr. Pons received the National Research Prize of the Spanish Biophysical Society in 2000 and has served as president of the NMR discussion group of the Spanish Royal Society of Chemistry (GERMN since its inception in 2001.

Ernest Giralt was born in 1948. He earned his first degree in 1970 and his Ph.D. in 1974, both from the University of Barcelona. After postdoctoral work at the University of Montpellier, France, he returned to Barcelona as an Assistant Professor. He was subsequently promoted to Associate Professor in 1977 and to Full Professor in 1986. He was Visiting Professor at the University of California, San Diego, and Research Associate at the Scripps Research Institute, USA, in 1991. Professor Giralt's main research interests lie in the fields of molecular recognition, peptide synthesis, and structure determination, in particular using nuclear magnetic resonance spectroscopy to study molecular-recognition processes. He has published over 290 papers and review articles as well as two books. In addition to being a founding member of the European Peptide Society, Dr. Giralt is Editor of *The Journal of Peptide Science* and serves on the editorial board of several other journals. He received the Narcis Monturiol prize in 1992, the Leonidas Zervas award in 1994, the Research Chair of Distinction from the Generalitat of Catalonia in 2001, the NMR prize from the GRMN of the Spanish Royal Chemical Society in 2002, and the Research National Prize and Medal from the Spanish Royal Chemical Society in 2003.

1.2.2. Type III: Noncovalent Peptide Dendrimers

These dendrimers have amino acids or peptides that noncovalently modify the periphery of a non-

Miriam Royo received her Ph.D. degree from the University of Barcelona in 1994. She spent three years as a postdoctoral fellow in the Division of Bones and Mineral Metabolism at the Beth Israel Deaconess Medical Center—Harvard Medical School. From 1997 to 2001 she worked as a postdoctoral research associate in the Department of Organic Chemistry at the University of Barcelona. Since 2002 she has been director of the Combinatorial Chemistry Unit at the Barcelona Science Park. Dr. Royo has published over 40 peer-reviewed articles and four patents. Her research interests include biopolymers, dendrimers, peptides, foldamers, drug delivery, combinatorial chemistry, and new therapies for central nervous system diseases (such as Parkinson syndrome or schizophrenia) and diabetes.

Fernando Albericio was born in Barcelona, Spain, in 1953. In 1981 he received his Ph.D. in Chemistry from the University of Barcelona under the supervision of Ernest Giralt. Following postdoctoral work with Victor A. Najjar at Tufts University, Jurphaas van Rietschoten at the Université d'Aix-Marseille, and George Barany at the University of Minnesota (1981–1984), he returned to the University of Barcelona as an Associate Professor. From 1992 to 1994 he was Director of Peptide Research at Milligen/Biosearch (Boston). He rejoined the University of Barcelona in 1995, at which point he was promoted to Professor. Currently, he holds a double appointment as Professor at the University of Barcelona and Group Leader at the Barcelona Biomedical Research Institute in the Barcelona Science Park. His major research interests cover practically all aspects of peptide synthesis and combinatorial chemistry methodology, as well as synthesis of peptides and small molecules with therapeutic activities. He has published over 350 papers, several review articles, and 15 patents, and he has coauthored the books *Chemical Approaches to the Synthesis of Peptides* and *Proteins and Solid-Phase Synthesis: A Practical Guide*. He is currently Editor of the *International Journal of Peptide Research and Therapeutics* and a Councilor of the American Peptide Society. He received the Leonidas Zervas award from the European Peptide Society in 1994 and the Research Chair from the Generalitat of Catalonia in 2004.

peptidic framework (Figure 2d). It is well-known that the internal cavities of dendrimers can encapsulate molecules. Dendrimer cavities have been functionalized with various compounds,[5] including peptides.

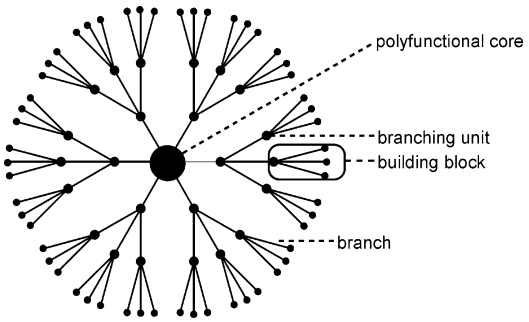

Figure 1. Schematic representation of a dendrimer. In this review, "building block" is used to signify both a branched unit and its branches.

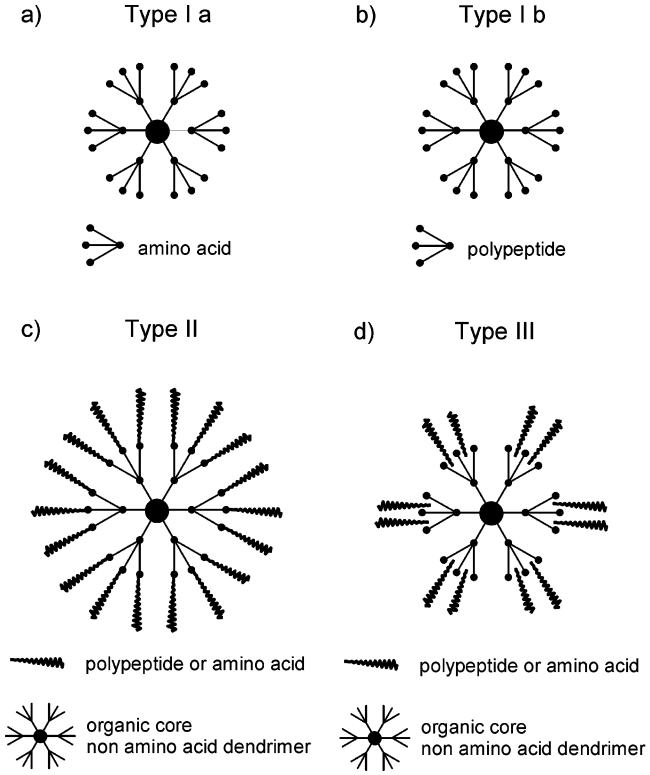

Figure 2. Different types of peptide dendrimers defined in this review.

In such cases, the structure of the dendrimer itself is nonpeptidic and the interaction between dendrimer and the amino acids or peptides depends on noncovalent interactions such as hydrogen bonds or ionic interactions.

1.3. Applications of Peptide Dendrimers

1.3.1. Why Peptides?

An important area of research in the field of dendrimer chemistry is the correlation of the function or application of a dendrimer with its architecture.[5–7]

The main characteristics of dendrimers are as follows: (i) monodispersity, (ii) a spherical shape, (iii) a highly branched architecture, (iv) a tightly packed surface, and (v) a high density of selected modifiable functional groups at their periphery.[1,8] As a result of their structure, dendrimeric macromolecules have unique properties that differ from those of linear polymers, such as viscosity,[9,10] thermal behavior,[11] or

molecular encapsulation.[12,13] For example, the nanoscopic dimensions of the dendrimeric catalytic carriers described by Rheiner et al.[14] allow the molecular dissolution and further elimination of reaction mixture by simple ultracentrifugation or dialysis techniques. Hannon et al. developed dendrimers that control ligand coordination through a designed cleft in a way analogous to that of proteins.[15] Moreover, dendrimers are used as vehicles for the delivery of drugs, DNA, or bioactive compounds to specific targets.[12,16−20] Although most dendrimers have not been initially proposed for pharmacological use, there are an ever-increasing amount of dendrimers with bioapplications.

The biological and therapeutical relevance of peptide molecules is obvious. They play an important role in diverse areas including cancer, antimicrobials, antivirals, the central nervous system, analgesia, asthma, allergy, and Ca^{2+} metabolism, among others.[21] Another interesting application of peptides is based on their ability to be taken up by cells, making peptides very useful for drug delivery.[22] One important drawback to the use of peptides in medicinal applications is their rather poor stability to proteases. In contrast to proteins, three-dimensional structures of peptides are often highly flexible and sensitive to environmental effects, but secondary structures of peptides can now be reasonably predicted in many cases.[23,24] Thus, successful structural design strategies have been developed for peptides. Merging the peptide and dendrimer fields was expected to produce synergistic effects. Features of peptide dendrimers include the following: (i) a protein-like globular structure which can act as a receptor by adapting to the shape of natural ligands; (ii) a polyvalent structure that enables simultaneous interactions between two or more ligands and receptors of the same type resulting in amplification of function; (iii) biocompatibility, which can minimize their cytotoxicity; (iv) water-solubility, which is crucial for polymers designed for systemic administration; (v) increased resistance to proteolysis, caused by their high degree of branching;[25] and (vi) biodegradability, which can circumvent problems related with polymer degradation.

There are expected similarities and differences between protein and peptide-dendrimer folding. Natural proteins have evolved to optimize the packing of their inner cores. This feature is generally absent in artificial dendrimers. On the other hand, local secondary structure is often stabilized by interactions between several peptide chains and may be enhanced in peptide dendrimers. Indeed, typical intermolecular stabilized structures, such as collagen triple helices or α-helical bundles, are further stabilized in dendrimer-like structures.[26,27] Flexible structural ensembles with a common secondary structure share some of the characteristics of molten globule structures but are conceptually different from them.[28,29] Crowding effects are known to shift equilibria toward the minimization of occupied space (i.e. supramolecular association or folding).[30−32] Peptide dendrimers are locally crowded structures, and their structure stabilizing properties should probably be viewed in this context. All of the aforementioned features make peptide dendrimers suitable compounds as protein mimetics, novel biomaterials for life sciences, and promising targets for biomedical applications.

1.3.2. Applications

Although the focus of this manuscript is the synthesis of peptide dendrimers, a brief review of their use in various biotechnological and biochemical applications is presented.[33,34] The reader is also referred to the review of Sadler and Tam,[35] which outlines the potential biomedical applications of multiple antigenic peptides (MAP), a special class of peptide dendrimers. The biocompatibility and immunocompatibility of polymeric materials are mandatory for their therapeutic utility.[36] Some potential polymer therapeutics[37] have been, or are being, withdrawn from the market due to adverse side effects.[38,39]

In medical diagnostics, peptide dendrimers have been used as contrast agents for magnetic resonance imaging (MRI), magnetic resonance angiography (MRA),[40,41] fluorogenic imaging,[42] and serodiagnosis.[43−47] In therapeutic applications, they have shown promise as vehicles for delivering drugs, DNA, peptides, or proteins.[41,48] Furthermore, dendrimers as multiple-armed macromolecular scaffoldings have found applications in the design of vaccines against bacteria, viruses, and parasites, as artificial enzymes.[49−54] or as inhibitors of infections,[55−58] inflammatory response,[59−62] autoimmune disease, or cancer metastasis.[63] More recently, peptide dendrimers have also been used to construct liposomes and biocompatible surfactants.[64] The supramolecular assemblies of dendritic dipeptides in solution possess porous structures[65] that may inspire diverse applications.

2. Synthesis

2.1. Peptide Synthesis

Peptide synthesis is based upon the proper combination and manipulation of temporary and permanent protecting groups, as well as the choice of efficient coupling reagents for the controlled formation of the peptide bond. Peptide synthesis can be carried out in solution, by using exclusively solid-phase methods, or by a hybrid solid-phase/solution approach. For a more comprehensive survey of the subject, the reader is referred to other articles.[66−68]

2.1.1. In Solution[69—72]

In solution-phase synthesis, reaction intermediates can be isolated and characterized at every step, greatly facilitating the monitoring of reaction progress and detection of side reactions during deprotection or coupling. Hence, undesired side products can first be removed before proceeding further with a given reaction. However, the isolation and characterization of intermediates is time-consuming, and the synthesis of even relatively small peptides may require a considerable investment of time and energy. The solution-phase synthesis of peptide dendrimers is an extension of the aforementioned concept in that long

reaction times and nontrivial purification steps are required. Furthermore, poorly soluble protected intermediates can jeopardize peptide synthesis.

2.1.2. On Solid-Phase[66,73,74]

In solid-phase methodology, developed by Merrifield,[75,76] reactions are driven to completion by the use of large excesses of reagents. The basic concept is the growth of a peptide chain, generally from the C-terminus, on an insoluble polymeric support, such as polystyrene cross-linked with divinylbenzene, functionalized in a suitable form. The general solid-phase peptide synthesis method (stepwise synthesis) involves the successive addition of N^{α}-protected amino acids (and, in the case of trifunctional amino acids, those with the side-chain functional groups protected as well) onto the N^{α}-unprotected amino group of the growing peptide chain anchored to the resin. Two double-protection strategies are commonly used in peptide synthesis: Boc (for N^{α}-amino groups) with Bzl (for side chains) and Fmoc (for N^{α}-amino groups) with tBu (for side chains). In the first strategy, Boc is removed with trifluoroacetic acid (TFA) and Bzl is removed by HF, which also cleaves the bond between the first residue and the solid support. In the second strategy, Fmoc is removed by piperidine and tBu is removed by TFA, which also cleaves the bond between the first residue and the solid support. After the incorporation of each protected amino acid, excess protected amino acids and coupling reagents as well as soluble side products are simply removed by extensive washing of the resin. The two major objections to the solid-phase approach are related to purification and characterization. Since the growing peptide chain is attached to an insoluble polymer, the purification and characterization of the various synthetic intermediates is not trivial. The two steps can only take place at the end of the synthesis, when the peptide has been detached from the polymer. This implies that the presence of deletion peptides formed by incomplete incorporation of the protected amino acid or removal of the temporary protecting group, which are closely related in structure to the target peptide, can make the final purification very tedious.

An interesting variation of the stepwise synthesis is the solid-phase convergent approach,[77] in which the construction of the peptide chain is carried out on solid-phase via coupling of protected peptide fragments prepared on solid-phase as well. The Boc/Bzl strategy is used with a fluorenylmethyl linker[78] from which the protected peptide is detached using piperidine, while the Fmoc/tBu strategy incorporates a chlorotrityl resin[79] from which the protected peptide is detached with a mild acid (1% TFA, hexafluoro-2-propanol, or HOAc). This approach allows the purification and characterization of the different protected peptide fragments prior to their coupling on solid-phase, thus providing a greater degree of homogeneity in the final compound. However, the incorporation of protected peptide fragments is difficult and usually requires a combination of the most efficient coupling reagents, such as those based on 7-aza-1-hydroxybenzotriazol (HOAt).[80]

Evidence of the limits of resin bead capacity has been observed in the convergent solid-phase preparation of polyproline-based dendrimers.[81] A high dendrimer-mass to bead-volume ratio could generate an enormous amount of tension on the beads, creating *stress* in the beads that would ultimately cause them to burst. This process can also be envisaged in terms of saturation, whereby a *saturated resin* would be analogous to a saturated solution. Therefore, special care should be taken in the solid-phase preparation of globular macromolecules such as those discussed in this review.

2.1.3. Solid-Phase/Solution Hybrid

Peptide dendrimers are generally prepared on solid-phase or by hybrid solid-phase/solution convergent approaches, which combine the advantages of solid and solution phases. In the hybrid approach, protected peptide fragments are first prepared on solid-phase and, once purified and characterized, are assembled in solution.[82−85] This approach takes advantage of the best characteristics of solid-phase synthesis, providing control of the synthetic process and allowing the incorporation of protected peptide fragments in solution. While the aforementioned factors are optimal for these types of syntheses, the use of powerful coupling reagents is nevertheless required.

2.2. Dendrimer Synthesis

Dendrimer synthesis generally includes the following steps: (1) selection of a suitable initiator that can be converted into a reactive initiator core in good yield; (2) definition of an iterative reaction sequence whereby the reactive initiator core is exposed to appropriate reagents or other reactive (partially protected) branched molecules, thus leading to high-yield conversions of branched assemblies with specific molecular surfaces; (3) reiteration of these step-growth or chain-growth sequences to produce dendrimers possessing concentric generations of repeating units and branch junctures. These factors define the number of branch cells, which accumulate exponentially within areas known as "generation zones". The order in which the aforementioned steps are carried out can vary with the synthetic approach used.

Two conceptually different synthetic routes for the construction of high-generation dendrimers exist: the *divergent* and the *convergent* approaches. In both approaches, dendrimer generations are created by the iterative repetition of a sequence of reactions. The growth of each generation requires both the activation of the growing dendritic fragment and the addition of a new monomer unit. The purity of the final dendrimeric product is related to the synthetic approach utilized. Dendrimers have been synthesized via a combination of the two methodologies. This strategy, referred to as the *double-stage convergent approach*, is more flexible and adaptable than either of its component methods.

2.2.1. Divergent Approach

The divergent approach (Figure 3) involves building the dendrimer outward from its core. The primary

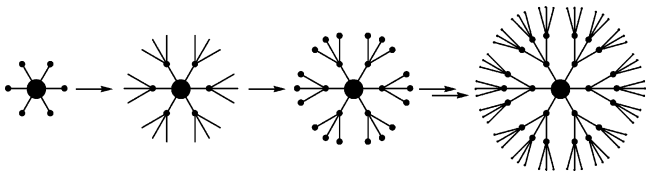

Figure 3. Divergent approach for dendrimer preparation.

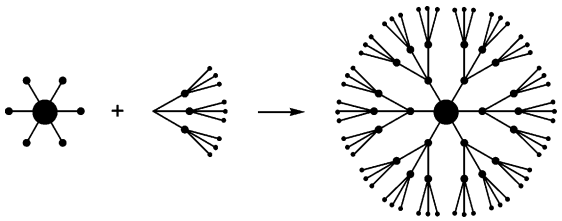

Figure 4. Convergent approach for dendrimer preparation.

drawback of this approach stems from incomplete reactions at dendrimer end groups, which can create structural defects that accumulate with each new generation. Chromatographic separation of the desired dendrimer is not always possible because byproducts often exhibit similar physical properties to those of the target molecule. Hence, higher generations of divergently constructed dendrimers always contain a certain degree of structural imperfection.[86] Poly(amidoamine) (PAMAM)[87] dendrimers[88−90] described by Tomalia, Newkome's "arborol" systems,[91] and poly(propyleneimine) dendrimers reported by Meijer[92] are examples of dendrimers constructed by a divergent approach.

2.2.2. Convergent Approach

The convergent approach begins from the dendrimer periphery and progresses inward toward the core. A segment growing with each reaction step is coupled to only one branching unit (Figure 4). While this approach facilitates the removal of undesired byproducts, for example, by gel permeation chromatography (GPC), steric hindrance in the reaction between segments and the growing core molecule limits the number of generations possible as compared to the case of the divergent method. Examples of dendrimers synthesized by convergent approaches are Fréchet's aromatic polyether dendrimers[93,94] and Moore's phenylacetylene dendrimers.[95−98]

2.2.3. Double-Stage Convergent Approach

Fréchet and co-workers have reported a double-stage convergent approach for the synthesis of dendrimers.[99−101] This approach involves the synthesis of building blocks using a divergent approach followed by a convergent dendrimer assembly. The strategy is based on the premise that AB_3 building blocks can be combined in parallel to yield homologous building blocks (Figure 5). These synthons can then be further combined in parallel to obtain higher generations. The procedure can be repeated to generate increasing numbers of dendrimeric fragments in a double-exponential manner. The double-stage convergent approach reduces the number of steps required for synthesis and purification, which makes the preparation of higher generation dendrimers less

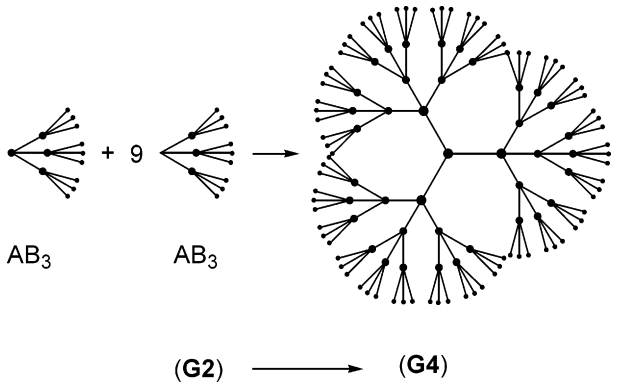

Figure 5. Double-stage convergent approach for dendrimer preparation.

tedious compared to traditional routes. Another advantage of the combined approach is the ability to modify building blocks, during their synthesis or the dendrimer assembly, with other buildings blocks to impart structural diversity.[99,101−104]

3. Types I and II: Covalent Peptide Dendrimers

3.1. Dendrimers Based on Amino Acids as Building Blocks

3.1.1. Multiple Antigenic Peptide (MAPs) System

It has been known since the early 1970s that synthetic peptides can induce antibody production in vivo by reacting with their cognate sequences in native proteins.[105,106] Such specific anti-peptide antibodies are useful in exploring biosynthetic pathways, in probing the structural functions of proteins, and in developing new synthetic vaccines.

Tam pioneered the synthesis and study of multiple antigenic peptides (MAPs), a distinct type of dendrimer.[35,43−47] MAPs contain a lysine dendrimer scaffold bearing multiple copies of an antigenic peptide, which enhance its immunogenicity. The work reported there was based on one of the earliest reports of a dendrimer, by Denkewalter et al.,[4] in which the synthesis of lysine dendrimers up to the tenth generation was patented. The basic idea consists of using a simple scaffold of trifunctionalized lysine as a low molecular weight synthetic carrier to which multiple copies of peptide antigen are bound (Figure 6). There are three main structural features of a MAP molecule: (1) a simple amino acid such as glycine or β-alanine used as an internal standard for the monitoring of the synthetic process; (2) an inner oligolysine core; and (3) multiple copies of synthetic peptide antigens. MAPs with two, four, eight, or sixteen copies of synthetic peptide antigens can be produced by utilizing oligolysine cores with one to four sequential levels of lysine residues. Although the higher level analogues are difficult to prepare, they are very useful because they significantly improve immunogenicity.[107] Some of the advantages of using MAPs as immunogens include simplicity in design and synthesis, versatility for various immune responses, reliability of generating site-specific antibodies, and generation of site-specific antibodies in the laboratory. The use of convergent synthesis based on a

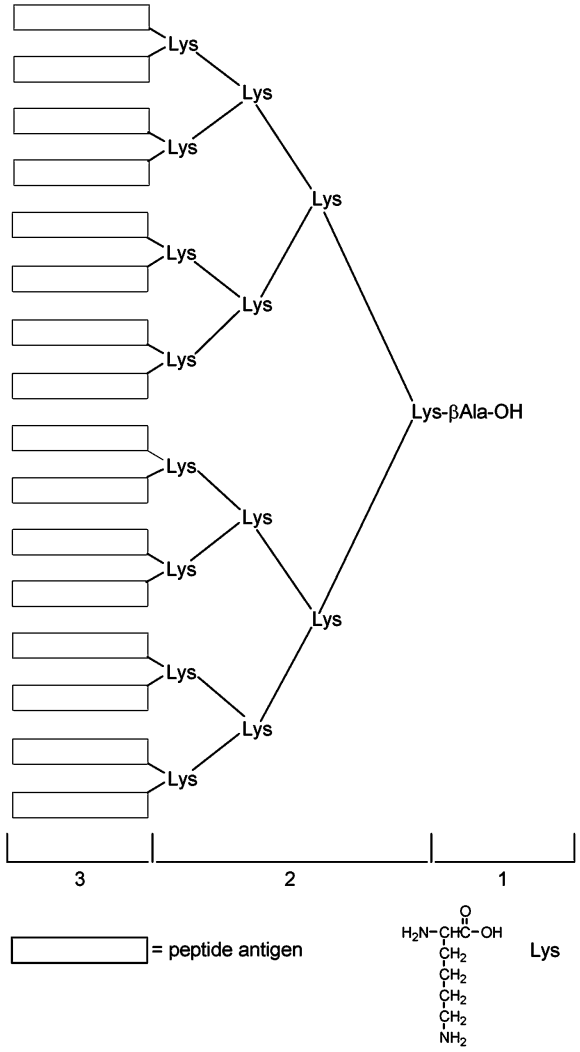

Figure 6. Schematic representation of a hexadecavalent MAP, a type Ia dendrimer. Reprinted with permission from ref 114. Copyright 1999 Wiley-VCH.

chemoselective ligation approach, which allows the use of unprotected peptide segments instead of bulky protected peptides, has improved the preparation of MAPs.[35,108,109] The MAP concept has also been extended to multiple antigenic glycopeptides in which L-lysinyl or L-ornitine cores are used as templates to attach glycopeptide antigens.[110−113]

Apart from their use in vaccine design, the multimeric constitution of MAPs has garnered interest in other areas.[33,114−121] MAPs can be divided into several classes according to their application: as immunoassay and serodiagnosis reagents,[122] inhibitors,[123] epitope mapping reagents,[124] artificial proteins,[125] protein mimics,[126] intracellular delivery agents,[127−132] supramolecular gels,[133−135] and purification reagents.[136]

As MAP scaffolds are based on lysine, which contains two points of reactivity (N^{α} and N^{ϵ}), polylysine molecules can be prepared using a solid-phase approach via Fmoc-Lys(Fmoc) or Boc-Lys(Boc). A broad range of peptide dendrimers based on MAP scaffolds with a varying degree of branching have been synthesized: (i) K_2K,[43] (ii) di-K_2K,[45] and (iii) tetra-K_2K[44] (Figure 7). Di-K_2K MAP is one of the most widely reported scaffolds due to its facile synthesis on solid-phase. Furthermore, its degree of branching

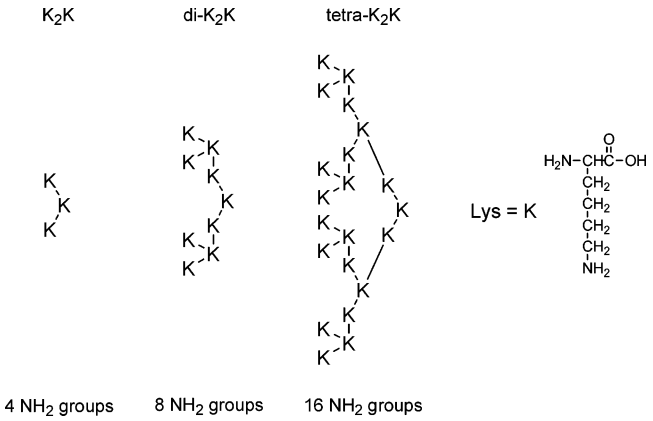

Figure 7. Polylysine scaffolds consisting of K_2K, di-K_2K, and tetra-K_2K.

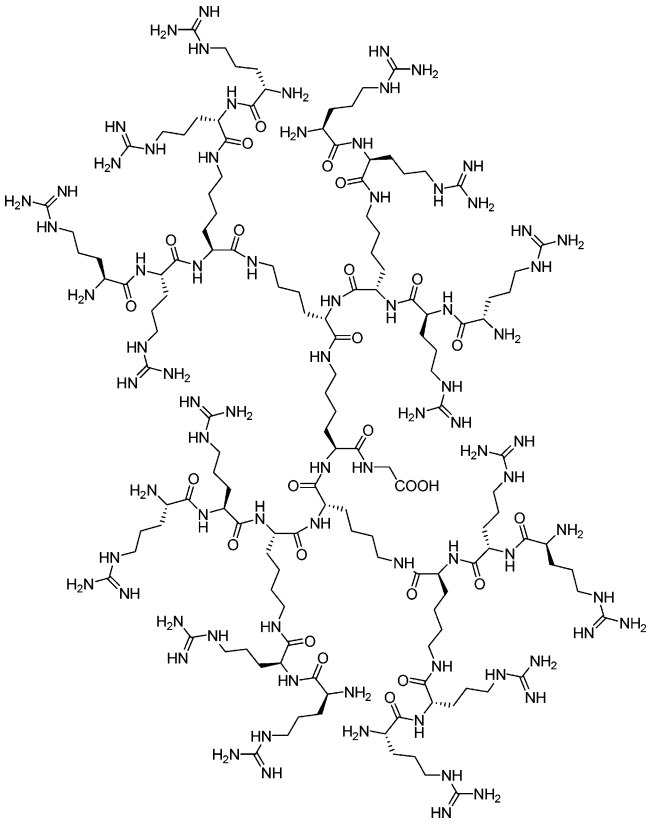

Figure 8. Structure of an arginine dendrimer (type Ia).

(eight reactive amine groups) is suitable for the introduction of a high number of peptide copies.

Arginine dendrimers have been designed and synthesized following the same synthetic approach used for polylysine MAPs.[137] These dendrimers consist of di-K_2K polylysine cores, L-lysine branching units, and L-arginine surface groups. Their synthesis involves an L-glycine linkage to a Wang polymeric support and employs an Fmoc-solid-phase approach (Figure 8). These dendrimers mimic the surface structure of endostatin and have been shown to interact with heparin, exhibiting a remarkable antiangiogenic activity.

These arginine-rich dendrimers have also been synthesized and used in the design of molecules for the intracellular delivery of exogenous molecules into cells.[138,139]

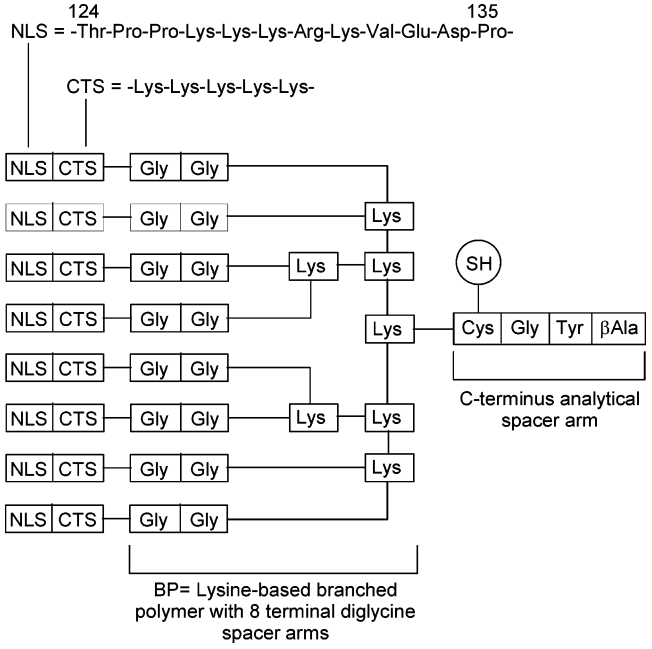

Figure 9. Structure of a loligomer, a type Ia dendrimer.

To exploit the ion transport properties exhibited by calixarenes, Xu et al. have recently reported an example of polylysine cores functionalized with different calixarenes. These compounds show promise in applications using nanostructured biological material.[140]

Loligomers are another type of peptide dendrimer based on polylysine MAPs. They function as multitasking peptide shuttles, which are capable of penetrating cells and self-localizing within cellular compartments. Each branch of a loligomer carries peptide signals that guide its transport and localization into cells.[141−147] A loligomer is generally composed of three different parts (Figure 9). The first part is a C-terminal region, or *analytical spacer arm*, consisting of three or five amino acids. In this part, the presence of a cysteine molecule allows the attachment of fluorescent labels or a biotin group. The second part, a *lysine-based branched polymer* (di-K_2K), is formed by eight identical *N*-terminal arms linked together. The presence of two glycine residues in each arm facilitates the linkage of the di-K_2K to the third part: a nuclear localization signal of the SV40 large T-antigen (NLS) and a cytoplasmic translocation signal consisting of a lysine pentapeptide (CTS) located on each *N*-terminal. Loligomers are prepared by solid-phase Boc chemistry using phenylacetamidomethyl (PAM) as solid support. These compounds have found applications as nonviral, nonlipophilic intracellular shuttles for gene transfer.[141,142,144,145]

Finally, an interesting variation of the classical polylysine MAP concept was developed by Park and co-workers, in which biocompatible cationic barbell-like triblock copolymers were synthesized and examined as potential gene carriers through their self-assembly with plasmid DNA.[148] The dendrimeric copolymers were synthesized in solution using a divergent approach and poly(ethylene glycol) as a core unit (Figure 10).

3.1.2. Polyglutamic Dendrimers

Although peptide dendrimers with molecular weights as high as those of a small protein have been reported, their exact three-dimensional shape in the solid state remains undefined due to the absence of structural information, such as single-crystal X-ray diffraction analysis. With the aim of designing simple models for globular protein mimics, Ranganathan et al. synthesized and investigated a collection of poly-

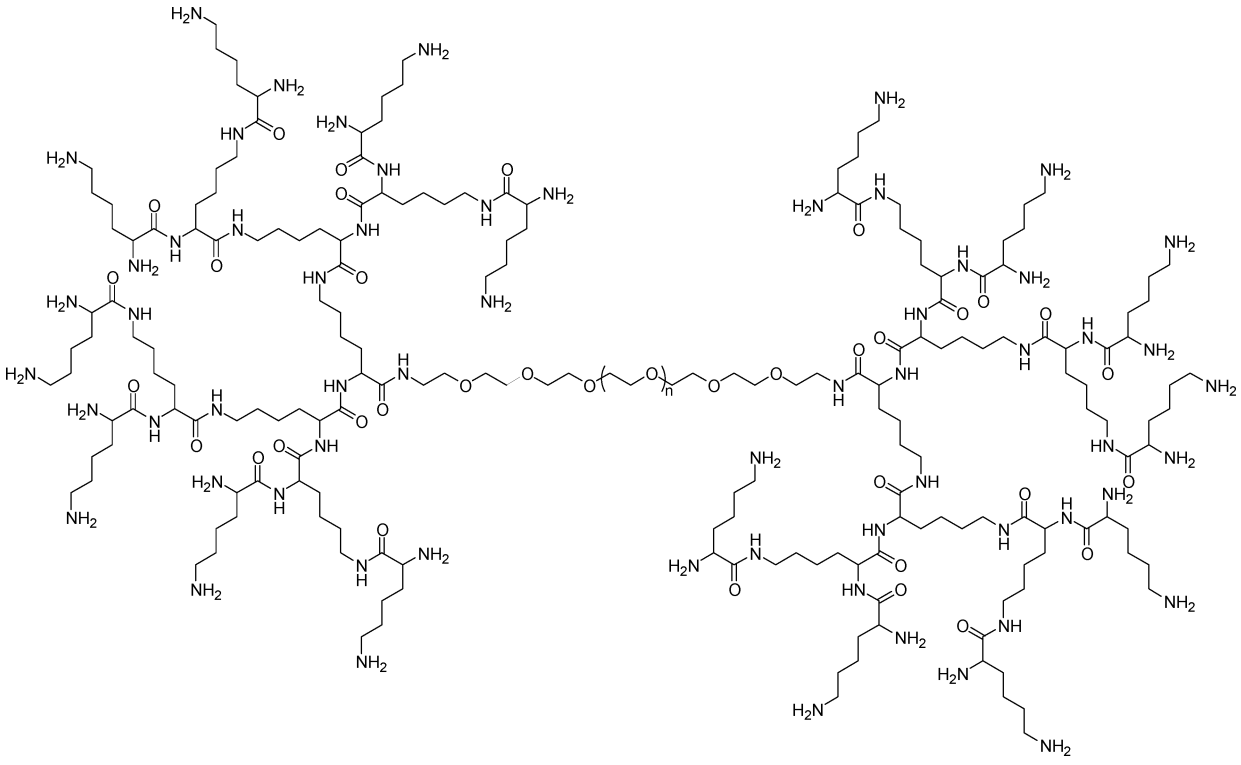

Figure 10. Structure of a type Ia dendrimeric copolymer based on poly(ethylene glycol) and polylysine.

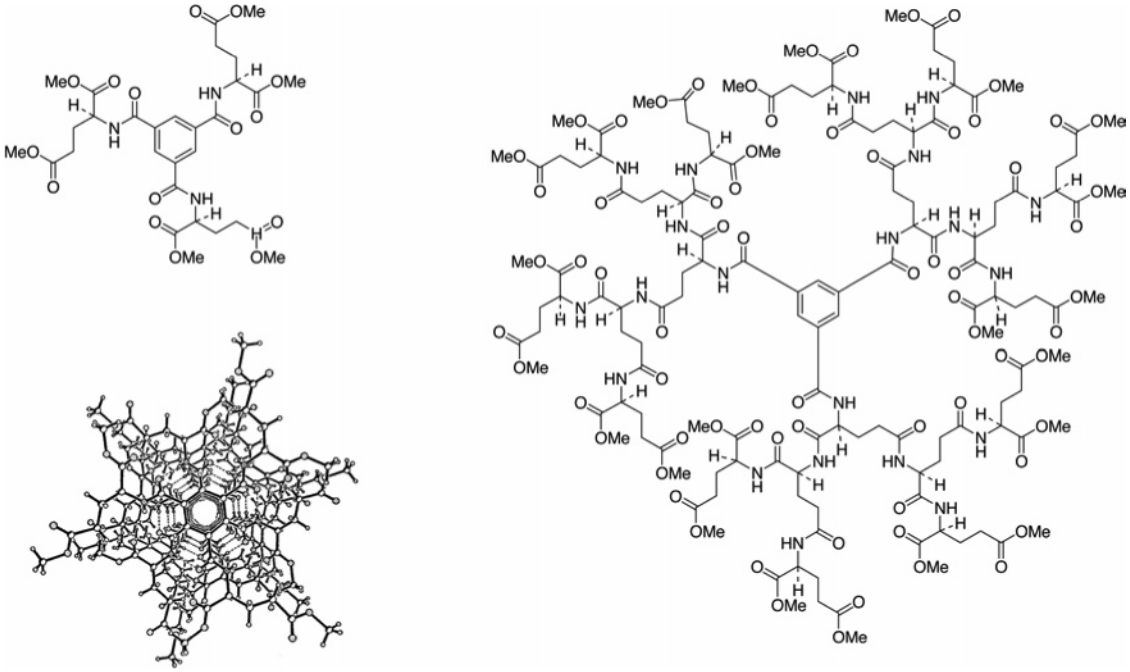

Figure 11. Structures of type Ia polyglutamic dendrimers and supramolecular $\pi-\pi$ stacking of triglutamyl-1,3,5-benzenetricarboxamide as observed in the crystal structure. Reprinted with permission from ref 151. Copyright 2000 Wiley, Inc.

glutamic dendrimers of various generations.[149−151] A 1,3,5-benzenetricarbonyl unit was used as a central core in order to induce cylindrical spatial arrangement of the dendrons by virtue of its threefold symmetry. In addition, the authors propose that this unit may also promote intermolecular association through either $\pi-\pi$ stacking or amide−amide hydrogen bonding. The trifunctional amino acid glutamic acid was chosen as the monomeric unit for assembling the matrix.

Ranganathan et al. reported the synthesis of first- and third-generation dendrimers, represented by the general structure AB_3 (A = 1,3,5-benzenetricarbonyl unit; B = Glu-diOMe or Glu-Glu_2-Glu_4-octaOMe), and demonstrated by X-ray crystallography that the first generation prototype assembles in the solid state into a cylindrical supramolecular stack of phenyl rings stabilized by contiguous NH···OC hydrogen bonding between the monomeric subunits.

Polyglutamic dendrimers were prepared in a single step using a convergent approach by treating commercially available 1,3,5-benzenetricarbonyl chloride with 3 equiv of either Glu-diOMe or preassembled Glu-Glu_2-Glu_4-$(OMe)_8$ in CH_2Cl_2 in the presence of triethylamine (Figure 11). Similar results were obtained with polyglutamic dendrimers using an oxalyl unit as a dendrimeric core.

Another example of polyglutamic dendrimers was described by Vinogradov,[152] in which a porphyrin moiety was used as a central core in dendrimers evaluated for the optimization of porphyrin-based phosphors for in vivo oxygen measurements. A series of Pd porphyrins were synthesized in order to study the quenching of phosphorescence by oxygen. It was shown that the polyglutamic branches adopt either open or compact conformations, depending on the solvent. The conformational transition of the glutamic chains alters the values of the quenching constants, thus changing the barrier to diffusion of oxygen toward the porphyrin core.

The Pd porphyrins of general formula PdPorph-Glu^NOR (where N is the number of the dendrimer generation) were synthesized by a divergent approach (Figure 12) using dicyclohexylcarbodiimide and pyridine in DMF to form the amide bonds.

A strategy for the total synthesis of chirally pure L-glutamic acid monomer-based dendrimers and their subsequent immobilization onto chromatographic supports was developed by Mitchell et al.[153,154] with the aim of obtaining new chiral HPLC stationary phases. The dendrimers were synthesized (Figure 13) using a convergent approach, employing L-glutamic acid ethyl ester and (*Z*)−L-glutamic acid as building blocks and HBTU and HOBt as coupling reagents. Dendrimer immobilization was accomplished by coupling the carboxy-derivatized dendrimers to aminopropyl-functionalized silica gel.

3.2. Dendrimers Based on Polypeptides as Building Blocks

3.2.1. Valine, Leucine, and Depsipeptide Dendrimers

Zimmerman and co-workers recently disclosed a general method for the preparation of monomers containing natural amino acids as well as of their corresponding third-generation peptide dendrimers.[155] A high-yielding cyanoethylation−hydrogenation procedure was employed to prepare simple AB_2 monomers from natural amino acids on a poly(ethylene glycol) (PEG) resin. The synthetic utility of their approach was assessed using L-valine and L-leucine (Figure 14), amino acids which have relatively bulky side chains. The authors suggest that the structural resemblance of these dendrimers to proteins, as well as their amenability to library

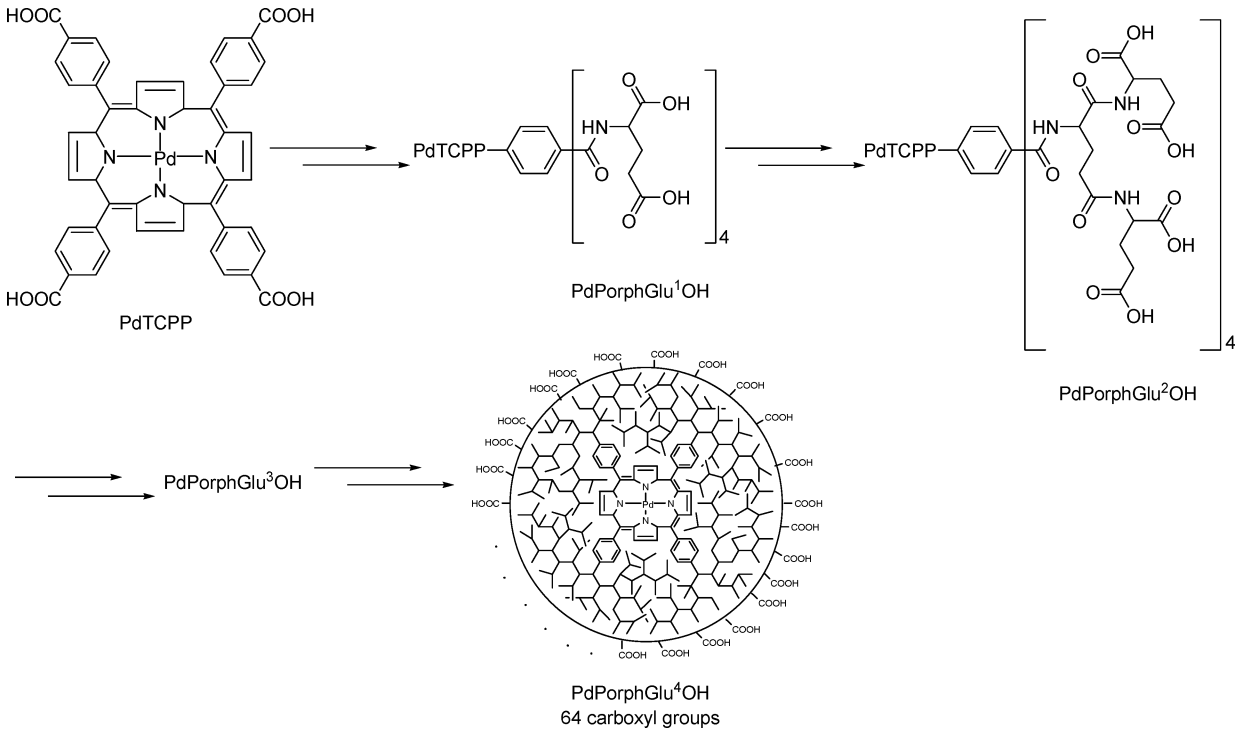

Figure 12. Divergent synthesis of dendritic polyglutamic Pd porphyrins, type Ia dendrimers. Reprinted with permission from ref 152. Copyright 1999 Wiley-VCH.

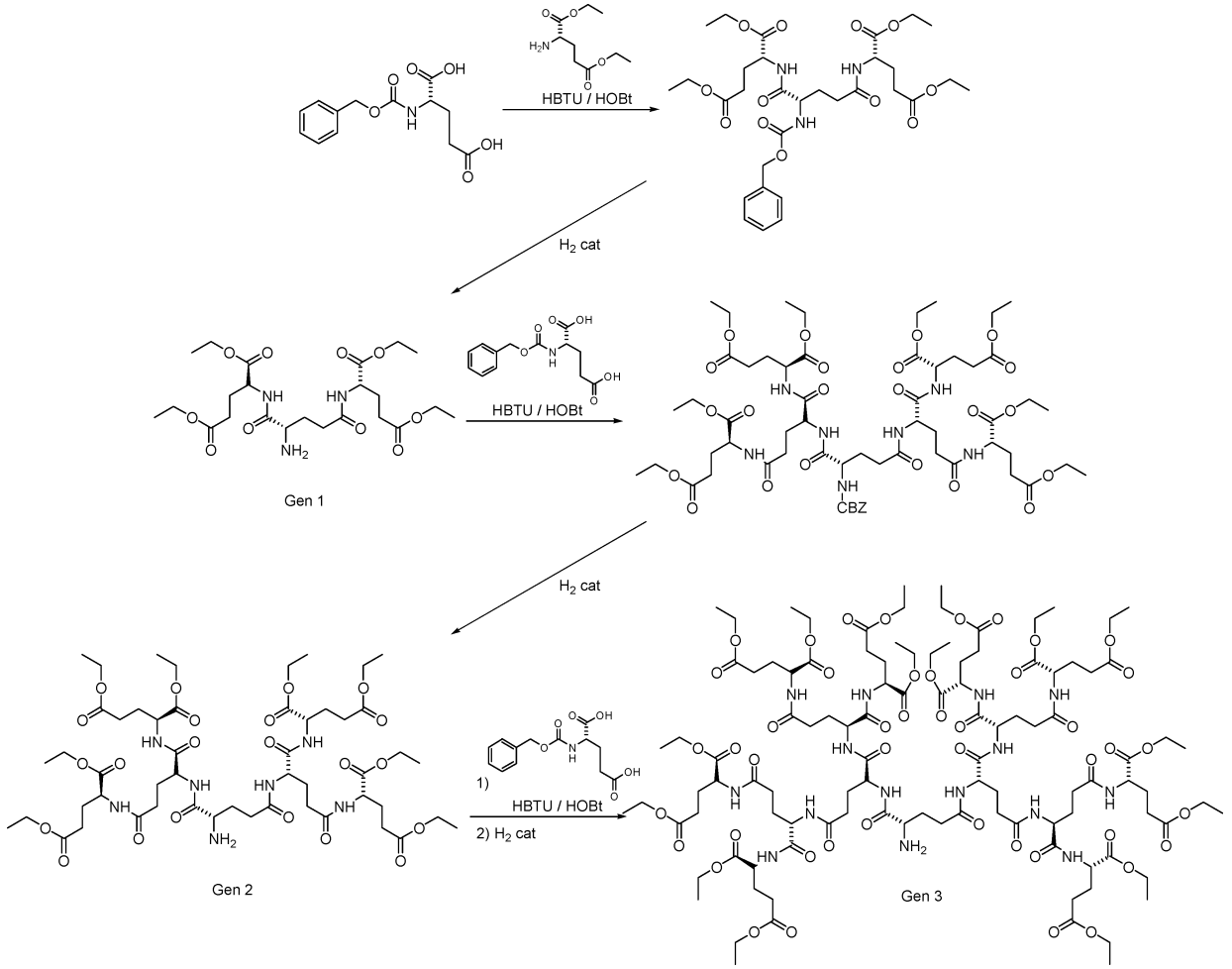

Figure 13. Synthesis of generation 1, 2, and 3 of L-glutamic acid ethyl ester type Ia dendrimers. Reprinted with permission from ref 154. Copyright 2001 The Royal Society of Chemistry.

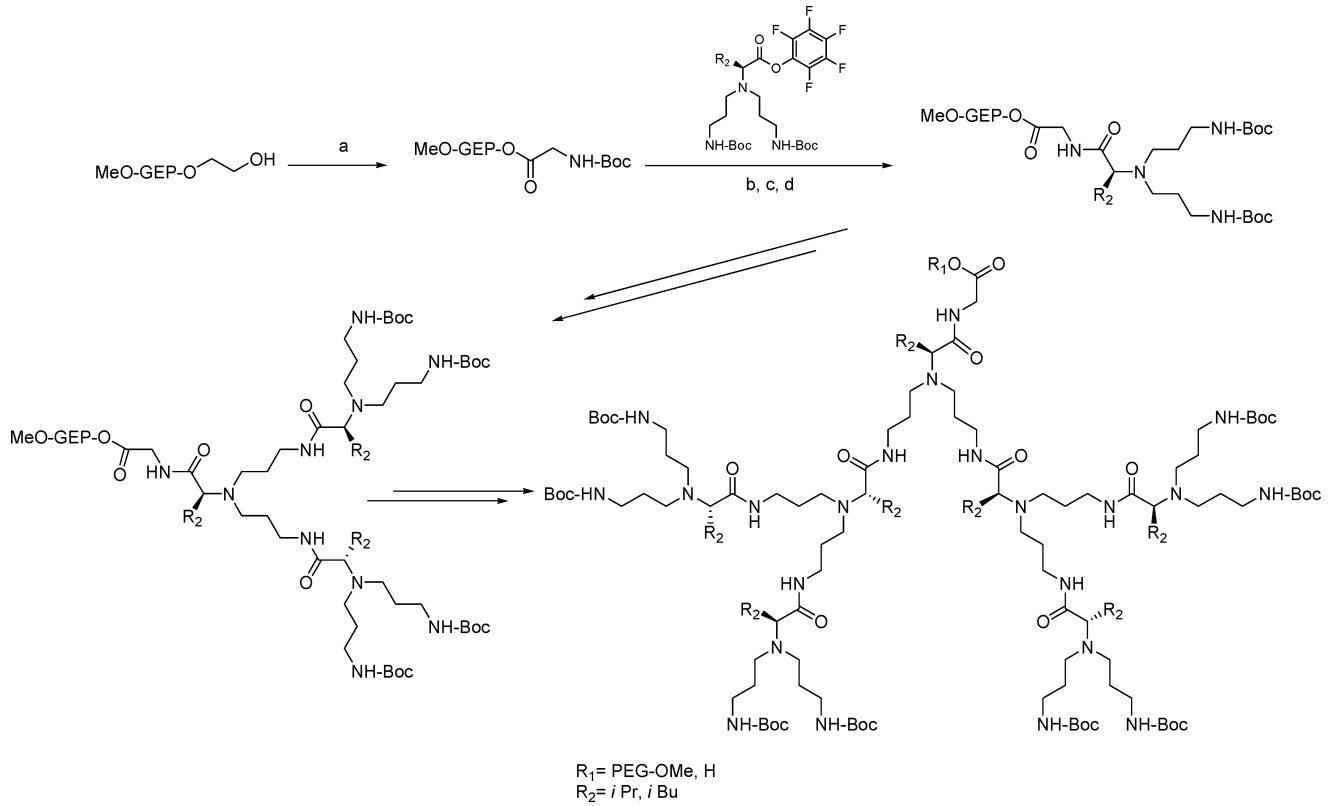

Figure 14. Synthesis of L-valine and L-leucine type Ib dendrimers: (a) Boc-Gly-OH, DCC, DMAP-DCM; (b) TFA-DCM; (c) active ester, HOBt, DIEA, DMF-DCM; (d) Ac_2O, DIEA-DCM. Reprinted with permission from ref 155. Copyright 1999 Wiley-VCH.

synthesis, may provide a novel approach to the discovery of new biomaterials.

Hirsch and co-workers developed new chiral dendrimers known as depsipeptide dendrimers, owing to their structural resemblance to depsipeptides.[156] Schemjakin coined the term "depsipeptides" to describe a class of natural products that consist of α-hydroxy and α-amino acids that are connected by ester and amide linkages, respectively.[157] These dendrimers consist of tartaric acid moieties as branching units and peptides as spacers. Their synthesis starts from (*R*, *R*)-, (*S*, *S*)-, and *meso*-tartaric acid as branching units and di- or tripeptides consisting of glycine, L-alanine, or L-leucine as chiral-spacer building blocks (Figure 15). A convergent approach was employed to obtain dendrimers up to three generations by using different combinations of stereoisomeric building blocks.

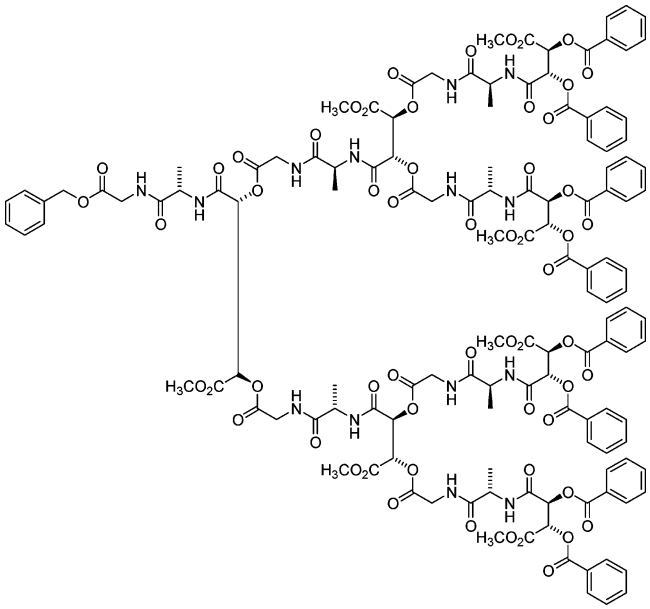

Figure 15. Structure of a third-generation depsipeptide type Ib dendrimer.

3.2.2. Polyproline Dendrimers

Proline is singular among the 20 genetically coded amino acids in that it contains both a cyclic backbone and a secondary, as opposed to a primary, α-amino group. These structural features impart unique stereochemical properties to proline. Polyproline oligomers exist in two distinct conformations. In organic solvents, they adopt a conformation known as polyproline I (PPI), a right-handed helix in which all peptide bonds are cis-oriented ($\omega = 0°$).[158] In aqueous solvents, they adopt the conformation known as polyproline II (PPII), a left-handed helix in which all peptide bonds are trans-oriented ($\omega = 180°$).[159] The transition from PPI to PPII implies a considerable increase in the length dimension of the helix that changes from 1.9 to 3.1 Å per residue.

The synthesis of peptide dendrimers based on polyproline helices,[160] in order to explore the conformational plasticity of polyproline chains in modulating dendrimer properties by changing branch length, has been reported. It was theorized that drugs could be stored inside of the dendrimers in organic solvents, where polyproline chains adopt a PPI conformation, and subsequently released under physiological condi-

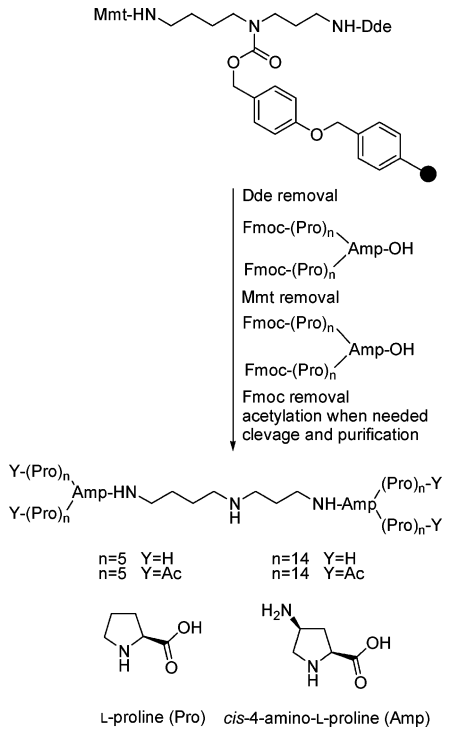

Figure 16. Solid-phase synthesis of type Ib polyproline dendrimers using a spermidine core.

tions as polyproline spacers extended to form PPII helices. An efficient protocol was established for the preparation of polyproline dendrimers based on a convergent solid-phase synthesis.[161,162] Pure Fmoc-Pro$_n$-L-Amp(Fmoc-Pro$_n$)-OH (n = 5, 14)[161] (where Amp symbolizes an aminoproline residue in order to maintain the structural coherence with the rest of the sequence and acts as a branching unit) building blocks were assembled on two different orthogonally protected cores: spermidine (Figure 16) and cyclic Lys-Lys (2,5-DKP) (Figure 17) using the highly efficient coupling agent PyAOP. In addition to being a novel method for the synthesis of other peptide dendrimers, this approach also demonstrates the suitability of convergent solid-phase synthesis for the preparation of these compounds.

Taking into account that the presence and interconversion of PPI and PPII can be analyzed by CD spectroscopy,[163−165] polyproline building blocks of different lengths, as well as their resulting dendrimers, were studied for their ability to adopt both helical conformations. It was observed that while all branched polyproline peptides can adopt a PPII conformation in H_2O, only the longer peptides (n = 14 and n = 19) form PPI helices.

Fluorescence spectroscopy experiments proved that polyproline dendrimers and ciprofloxacin interact in alcohol. Finally, it was shown that endothelial rat kidney cells actively internalize labeled forms of polyproline dendrimers. The aforementioned results demonstrate the feasibility of using polyproline dendrimers as new drug delivery systems.

Another example of proline-containing dendrimers has been described by Goodman et al., in which 162-residue collagen-mimetic dendrimers exhibit enhanced stability compared to that of equivalent scaffold-terminated structures.[166] Collagen, the main

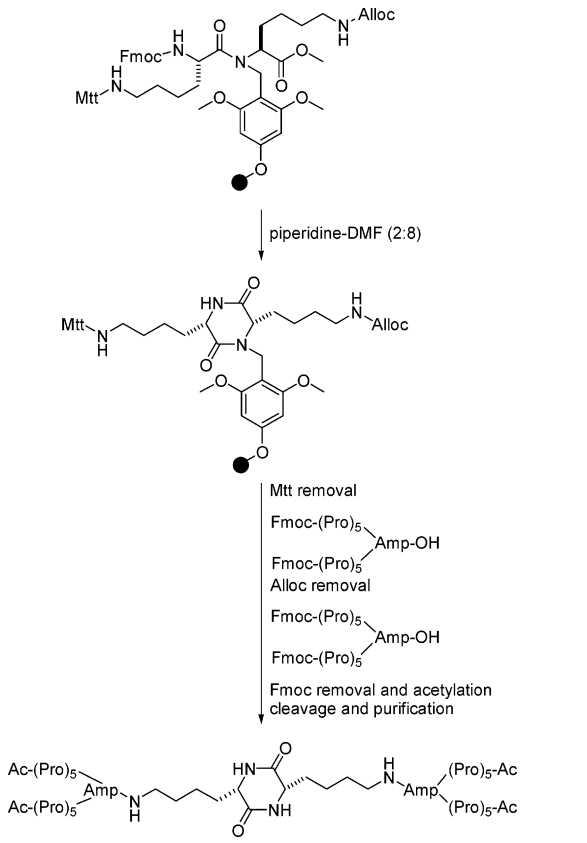

Figure 17. Solid-phase synthesis of type Ib polyproline dendrimers using a cyclic Lys-Lys (2,5-DKP) core.

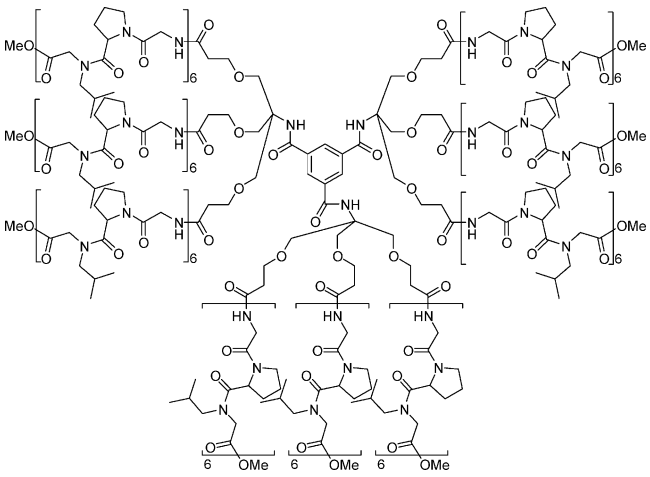

Figure 18. Structure of a collagen-mimetic type Ib dendrimer.

structural protein in mammals, consists of triple helical peptides composed of Gly-Xaa-Yaa trimer repeats.[167,168] The authors reported the synthesis and conformational properties of collagen-like dendrimers built from Gly-Pro-Nleu and Gly-Nleu-Pro sequences (where Nle denotes *N*-isobutylglycine) and *N*-(benzyloxycarbonyl)tris(carboxyethoxymethyl)aminoethane (Z-TRIS[OH]$_3$) as a scaffold to assemble collagen mimetics. The Z protecting groups on the scaffold-terminated peptides were removed and allowed to react with trimesoyl chloride to create the dendrimeric structures (Figure 18).

The triple helicity of all structures was determined by thermal denaturation and monitored by optical

rotation and circular dichroism. The spectroscopic studies were carried out in H_2O as well as in the triple-helicity-enhancing solvent mixture ethylene glycol (EG)/H_2O (2:1, v/v).[169] The optical rotation data indicate that the molecules studied exhibit a cooperative melting transition in H_2O while analogous single-chain compounds do not. A shallow transition for the single-chain molecules can be seen in EG/H_2O (2:1), indicating a minor formation of triple helices.

3.2.3. Amphiphilic Dendrimers

"Spider-web amphiphiles", a novel category of two-dimensional lipid clusters in which dendrimers contain amphiphilic structures in every unit, were synthesized and named by Kikuchi et al.[170] Amphiphilic units based on a Lys-Glu-Lys tripeptide with hydrophobic tails at the C-terminal and a polar head at the N-terminal were connected through stepwise peptide coupling. This structural design allowed the separate introduction of the polar head and hydrophobic tails. Branching and expansion of the web structure were achieved by stepwise deprotection and coupling between the Lys and Glu residues, utilizing a convergent strategy based on conventional solution-phase peptide chemistry. The synthesis of the spider web was carried out to three generations in three polar head/hydrophobic tail combinations: acetyl head/C_{16} chain, acetyl head/C_{18} head, and ammonium head/C_{16} chain. All of the spider webs exhibited expanded monolayers except for the C_{18}-chain amphihile. These spider-web amphiphiles can accommodate/encapsulate lipid components according to a stoichiometric rule whereby the number of accommodating guest lipids is consistent with the number of branching points in the host compound. These compounds may be useful for the development of a membrane-based delivery system for lipophilic drugs.

3.3. Polypeptides Grafted onto Organic Cores

Bradley and co-workers developed an efficient divergent solid-phase synthesis of PAMAM dendrimers to produce homogeneous molecules.[171] They also showed that the dendrimeric material could be conveniently functionalized while on the solid support, thus providing a route to a myriad of functionalized dendrimers that would benefit from the advantages of solid-phase synthesis. The primary aim of their work was to obtain high-loading resin beads for combinatorial chemistry.[172−175] Furthermore, these products were shown to be suitable starting points for the solid-phase synthesis of peptide-modified dendrimers up to six amino acid residues in length.[171] Thus, these PAMAM dendrimers can be considered as scaffolds onto which peptides can be assembled at their amino terminal functionalities.

Peptide dendrimers have been designed and synthesized as artificial photosynthetic systems,[176−178] whereby amphiphilic α-helix peptides were introduced at the end groups of PAMAM dendrimers. The compounds were shown to bind one molecule of metalloporphyrin for every two α-helices to form a metalloporphyrin array. Their electron-transfer functions increased with each generation of the dendrimer, a phenomenon that was attributed to the packing density of the metaloporphyrins resulting from the dendrimer architecture.

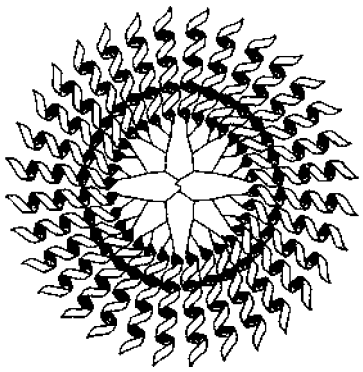

Figure 19. Schematic representation of a PAMAM type II dendrimer covalently modified with helical peptides. Reprinted with permission from ref 182. Copyright 2000 The Royal Society of Chemistry.

A direct method for the formation of peptide and carbohydrate dendrimers has been successfully developed in which PAMAM dendrimers are covalently modified via a convergent approach with either peptide or carbohydrate molecules.[179,180]

Other examples of periphery modification of dendrimers with peptides have been shown by Higashi et al.,[181,182] in which helical polyglutamic peptides where assembled on a PAMAM dendrimer surface by graft polymerization (Figure 19). The resultant peptide dendrimers were shown to have enhanced peptide-segment helicity as compared to that for nondendrimer based analogues, a fact attributed to aggregation of the peptide segments on the dendrimer surface. Moreover, the peptide dendrimers were capable of interacting with aromatic α-amino acids, preferentially with D-enantiomers over L-enantiomers. The bound α-amino acids were not released into the water phase but were transferred into the inner core of the dendrimer even when a conformational change of the helix segment was induced by pH variation.[27]

3.4. Non-natural Amino Acid-Based Dendrimers

As previously mentioned, the formation of amide bonds in the synthesis of peptides and proteins is extremely well established and consequently has been used in the preparation of other types of molecules containing amide bonds. Recent examples of dendrimers containing non-natural amino acids as well as an example of depsipeptide dendrimers are described herein.

Chow et al. prepared a series of highly polar poly(β-alanine) dendrimers in solution (Figure 20). Proton NMR studies revealed that the poly(β-alanine)-based dendrimeric species have an open structure in polar aprotic solvents, which allows solvent molecules to freely interact with the interior. Due to the presence of a large number of carbamates and amide groups, the dendrimers act as H-bonding sponges, which strongly bind protic solvents such as water. In addition, they form intermolecular aggregates of nanoscopic size in both nonpolar and polar aprotic solvents.[183]

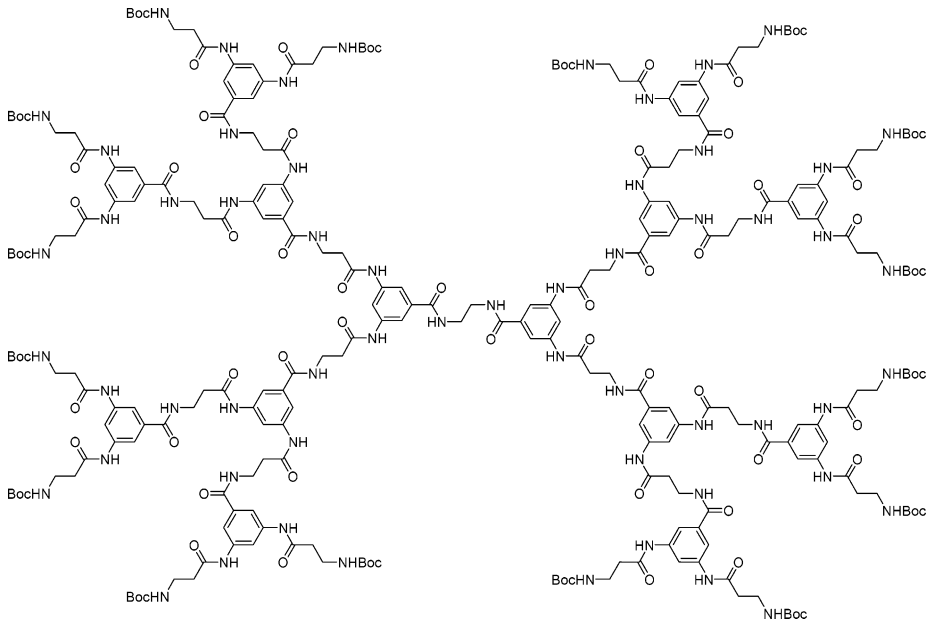

Figure 20. Structure of a third-generation poly(β-alanine) type II dendrimer.

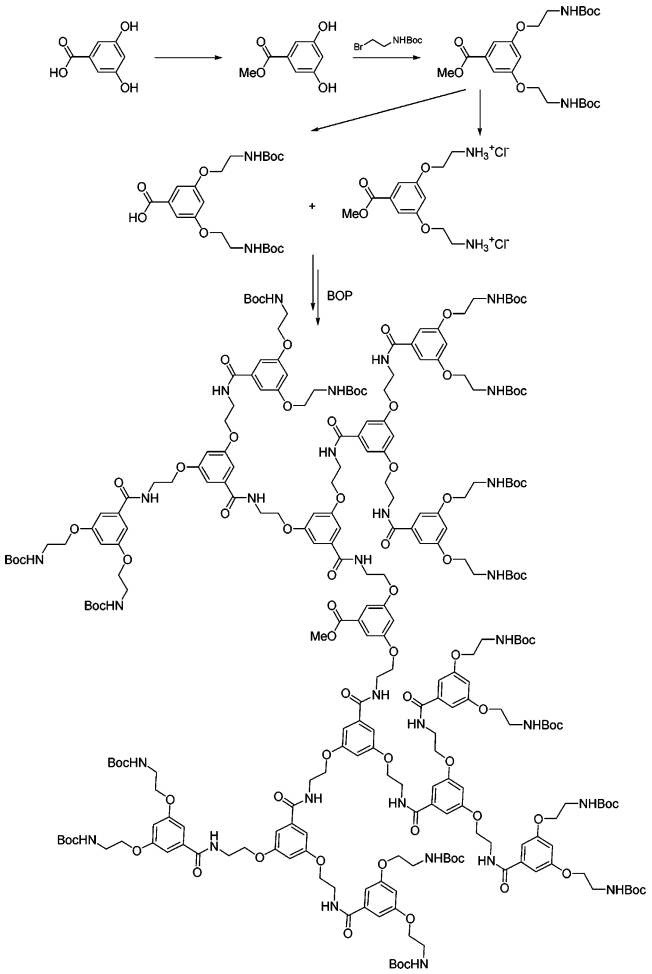

Figure 21. Synthesis of a fourth-generation, amino acid-based type II dendrimer containing aromatic residues. Reprinted with permission from ref 184. Copyright 1997 Elsevier.

Liskamp et al. have described the convergent synthesis of amino acid dendrimers containing aromatic residues[184,185] employing 3,5-bis(2-*tert*-butyloxycarbonylaminoethoxy)benzoic acid methyl ester,

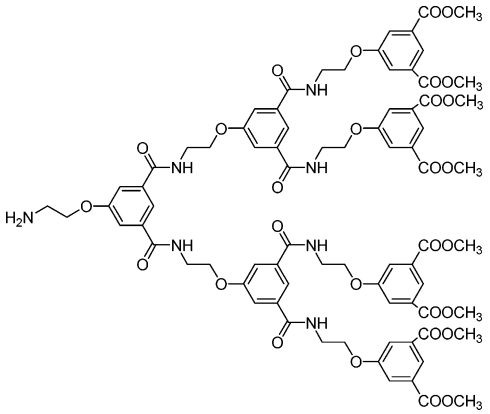

Figure 22. Structure of a third-generation dendron based on 5-(2-aminoethoxy)isophthalic acid.

which is easy accessible from 3,5-dihydroxybenzoic acid and 2-bromoethylamine hydrobromide. The methyl ester moiety was then converted to both the "surface" monomer, via saponification, and the "branching" monomer, via removal of the Boc group. The first dendrimer was subsequently obtained via BOP coupling of the two preformed units. The preparation of higher generations consisted of repeating the two-reaction cycle: saponification of the methyl ester and coupling to the branching monomer, affording dendrimers up to the fifth generation (Figure 21).

Furthermore, the exterior of these dendrimers was modified with catalytically active transition metal complexes in order to investigate their potential as molecular sensors.[186]

Perfectly branched polyamide dendrons based on 5-(2-aminoethoxy)isophthalic acid were synthesized following a convergent approach up to the fourth generation by Voit et al.[187] (Figure 22). Their aim was to synthesize perfect, sterically unhindered dendrimers by amide bond formation using aromatic–aliphatic building blocks. The preparation of these molecules involved the repeated reaction of 5-(2-*tert*-butoxycarbamylethoxy)isophthalic acid with a 2-fold

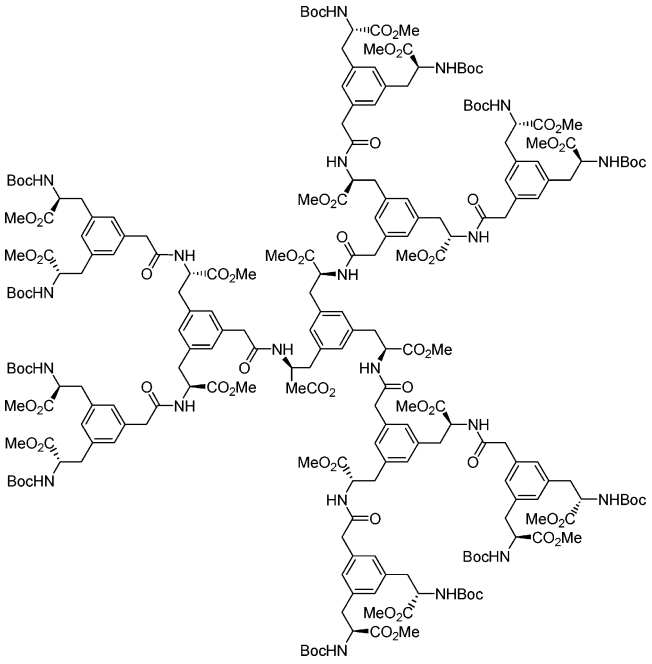

Figure 23. Structure of an aromatic amino acid type II dendrimer.

excess of 5-(2-aminoethoxyhydrochloride)isophthalic acid dimethyl ester or its higher generation analogues via classical activation and protection methods of peptide chemistry.

Other examples of amino acid dendrimers were described by Frejd. Second-generation dendrimers based on bis- and tris-aromatic amino acids were synthesized using a Heck-coupling/hydrogenation protocol and TFFH as coupling reagent for the amide bond formation[188,189] (Figure 23).

Carbohydrate and dendrimer chemistries were combined by Lindhorst et al. in order to design multivalent glycoconjugate mimetics to study and manipulate carbohydrate−protein interactions.[190] Orthogonally protected AB_2-type carbohydrate units were used as building blocks and iteratively assembled via peptide linkages to yield various generations of carbohydrate-containing dendrons in either a divergent or convergent manner (Figure 24).

4. Type III: Noncovalent Peptide Dendrimers

As previously mentioned, many of the typical physical properties of dendrimers, such as solubility, macroscopic shape, and multivalency, are determined by the nature of the peripheral groups. While most terminal group modifications of dendrimers are based on covalent bonds, the use of supramolecular interactions to obtain new dendrimeric peripheries is limited. Type III dendrimers acquire peptidic characteristics via integration of amino acids into a shell surrounding an organic framework. The specific interactions between a host dendrimer and a guest molecule involve both the core and the exterior of the dendrimer.

4.1. Poly(propyleneimine) Dendrimers with Peptides

An innovative example of this type III peptide dendrimer has been described by Meijer et al., in which a poly(prolyleneimine) dendrimer with 64 amine end groups was successfully modified with several natural amino acids such as L-Ala, L-Leu, L-Phe, D-Phe, and L-Tyr.[191] These macromolecules, named "dendritic boxes", were shown to be capable of physically locking or encapsulating guest molecules in their internal cavities when a dense shell was constructed in their peripheries (Figure 25). The authors demonstrated that the selective release of guests encapsulated in a dendritic box could be accomplished by a multistep process. Two guests that

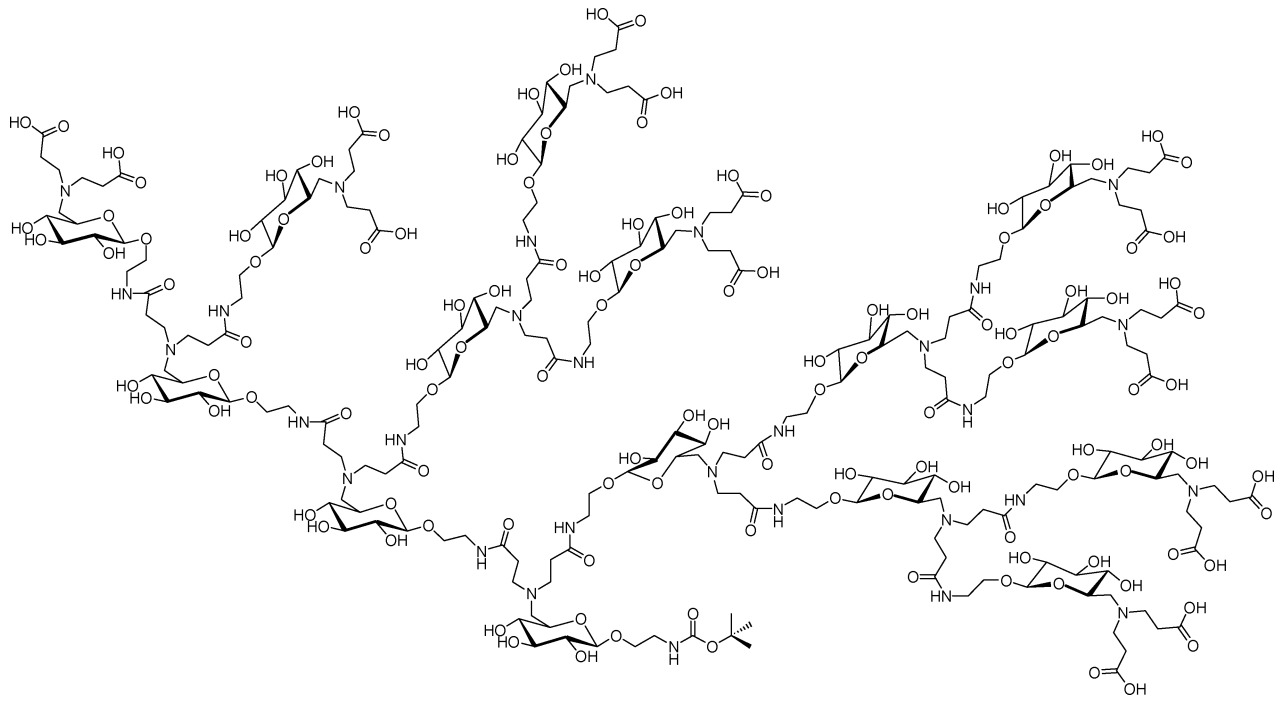

Figure 24. Structure of a third-generation glycopeptide dendron.

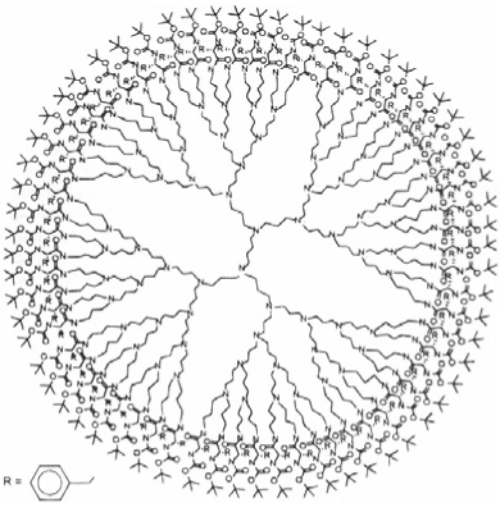

Figure 25. Structure of a "dendritic box".

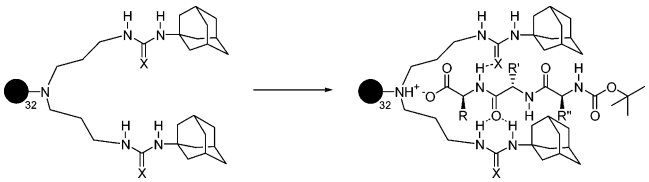

Figure 26. Proposed scheme for the formation of complexes between a dendrimer host and an *N*-terminal Boc-protected tripeptide (X = O, S) to yield a type III dendrimer. Reprinted with permission from ref 193. Copyright 2002 Wiley-VCH

differ in size are first encapsulated in the box, followed by a dialysis treatment to remove the adherent and excess guest molecules. The shell is then partially perforated, yielding a modified dendritic box in which only the larger guest is entrapped and from which the smaller guest is liberated. Subsequent removal of the shell liberates the larger guest, and the starting poly(propyleneimine) dendrimer is recovered.

Poly(propyleneimine) dendrimers have also been modified with peptides using a supramolecular approach. Adamantyl urea and adamantyl thiourea modified poly(prolyleneimine) dendrimers act as hosts for *N*-terminal Boc-protected peptides and form chloroform-soluble complexes[192,193] (Figure 26). The guest interacts with the dendrimer host through urea−urea or urea−thiourea hydrogen bonding as well as through ionic interactions between the outer shell tertiary amines of the dendrimer and the carboxylic terminus of the guest. Furthermore, the authors investigated the influence of steric repulsion between peptide side chains and the dendritic host on the formation of stable complexes between the peptide and the dendrimer.

Finally, the application of protein−protein self-assembly principles to dendrimer architecture has recently been reported in the formation of leucine zippers linked to a PAMAM core for selectively complexing helical peptides.[28,194] The authors utilized a maleimide-functionalized zero-generation PAMAM core allowing its chemoselective covalent linkage to cysteine-containing peptides. The peptides consisted of pH-sensitive cognate leucine zippers with hydrophobic cores and polar exteriors, which were modified with a cysteine linker at the C-terminus, thus enabling tetramer formation. The aforementioned example provides a suitable methodology for constructing novel dendrimer−peptide complexes with helical secondary structure.

5. Conclusions and Future Outlook

Polymer scientists have become increasingly interested in developing new materials for biomedical applications. Peptide dendrimers are especially attractive, since they exhibit characteristics particular to both dendrimers and peptides, thereby yielding macromolecules with a high structural similarity to globular proteins. Many natural proteins adopt spherical shapes in solution and are generally classified as globular proteins. This globular shape, which arose through millions of years of evolution, is also reflected in peptide dendrimers, whereby fractal geometry provides spherical macromolecules with a tight interior and a high surface density in outer layers.

Peptide dendrimers contain either natural or nonnatural amino acids in their framework. These amino acids can be incorporated into the different parts of the dendrimer (i.e the core, building blocks, or branching units) as part of peptide fragments used as building blocks or as peptides grafted onto the surface of an organic core through covalent linkages or noncovalent interactions.

Dendrimers can be synthesized using traditional approaches (convergent or divergent) as well as a modified approach (double-stage convergent). The presence of peptides confers proteinlike properties to dendrimers. In addition, the amenability of peptide synthesis to solution or solid-phase techniques facilitates the production of peptide dendrimers.

There exists great potential for structural diversity among this intriguing class of polymers. The production of vast combinatorial libraries of dendrimers, based on the 20 genetically coded amino acids as well as hundreds of noncoded amino acids, with variations in branching units or building blocks at each generation can be imagined. Such combinatorial libraries should promote the discovery of lead structures and products with novel properties.[52] Moreover, analysis of the libraries should facilitate exploration into new areas of application beyond those mentioned above.

As observed with type III dendrimers, the concepts of supramolecular chemistry have strongly impacted the dendrimer field. Noncovalent interactions are expected to be increasingly used for the hierarchical assembly of dendrimers of higher complexity or the introduction of supradendrimer structures via interaction between branches from different dendrimers, as demonstrated in the recent work of Zhou et al.[28]

Antibodies are naturally branched protein structures with a high degree of selectivity and affinity for certain antigens. The expected natural convergence between these natural and artificial dendrimers has already appeared in the literature.[195] Interaction with biological systems has been a recurring objective in dendrimer design, and the use of self-assembled, highly symmetrical, natural particles as dendrimer cores would open a path to the construction of very large dendrimer structures. The

structure of an icosahedral virus particle saturated with antibodies located in each of the 15 twofold symmetry axes of the particle is already known.[196] The highly symmetric particles could be additionally functionalized using methods described in this review, resulting in potentially useful nanomaterials.

In conclusion, the use of peptide dendrimers in the basic sciences as well as in fields such as therapeutics, tissue engineering, and environmental engineering may soon come to fruition. These promising biologically inspired macromolecules offer rich opportunities for investigation.[34,197,198]

6. Abbreviations

Alloc	allyloxycarbonyl
Amp	*cis*-4-amino-L-proline
BOP	benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
Dde	1-(4,4-dimethyl-2,6-dioxocyclohexylidine)ethyl
DKP	2,5-diketopiperazine
DMF	*N*,*N*-dimethylformamide
Fmoc	9-fluorenylmethoxycarbonyl
HBTU	1-[bis(dimethylamino)methylene]-1H-benzotriazolium hexafluorophosphate 3-oxide
HOBt	1-hydroxybenzotriazole
Mmt	4-methyltrityl
PAMAM	poly(amidoamine)
Phth	phthalimido
PP	polyproline
PyAOP	7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate
TFFH	tetramethylfluoromethylformamidinium hexafluorophosphate

7. Acknowledgment

Work in the authors' laboratories was partially supported by funds from CICYT (BQU2002-02047, BIO2002-2301, and BQU2003-00089) and the Generalitat de Catalunya (Grup Consolidat and Centre de Referència en Biotecnologia).

8. References

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CR030449L