PEPTIDE DENDRIMERS

Reported by Chao Wang February 28, 2005

INTRODUCTION

Dendrimers are novel macromolecules having well-defined hyperbranched structures. They generally consist of three unique structural components: a central core, polyvalent branching units, and surface functional groups attached to the termini of the branching units (Figure 1). Although Flory described the theoretical existence of dendrimers in 1941,[1] it was not until 1978 that Vögtle synthesized the first monodisperse branched molecule.[2] Newkome and Tomalia both reported the synthesis of branched molecules in 1985, naming these molecules arborols[3] and dendrimers[4], respectively. The high molecular weights possible with dendrimers, along with their monodispersity, and ability to be easily functionalized have enabled their use in a wide variety of applications in polymer science, catalysis and biology.

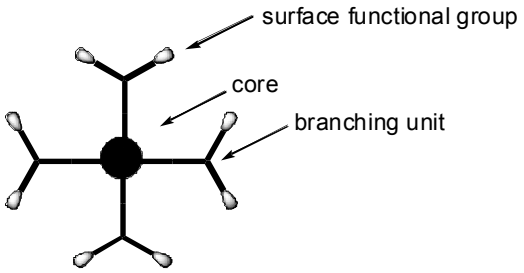


Figure 1. Structural features of a dendrimer

Peptide dendrimers are radially branched macromolecules that contain a peptidyl branching core and/ or peripheral peptide chains,[5] and they can be divided into three categories. One category consists of "grafted" peptide dendrimers, having peptides only as surface functionalities. The second category is peptide dendrimers that composed entirely of amino acids. The third are dendrimers utilizing amino acids in the branching core and surface functional groups, but having non-peptide branching units. Peptide dendrimers can be synthesized using either divergent or convergent approach, and the availability of solid-phase combinatorial methods enables large libraries of peptide dendrimers to be produced and screened for desired properties. Peptide dendrimers have been used in industry as surfactants, and in biomedical science as multiple antigen peptides (MAP), [5] protein mimics[6] and vehicles for drug and gene delivery.[7,8] Additionally, Reymond and coworkers have utilized peptide dendrimers as esterase catalysts.[9] This report will review the synthesis and corresponding applications of peptide dendrimers as immunogens, protein mimics, and catalysts in ester hydrolysis reactions.

DENDRITIC EFFECT ON MATERIAL PROPERTIES

In the early 1990s, Newkome and co-workers pioneered the field of gel-phase material formation from dendrimers.[3,10] More recently, Smith and co-workers developed poly(L-lysine) dendrimers which form gel-phase materials in nonpolar organic solvents.[11] Three dendrimers from the first to the third generation, **G1-SS-G1**, **G2-SS-G2** (Figure 2) and **G3-SS-G3**, were synthesized. The addition of methanol broke up the gel, indicating that hydrogen bonding is the main driving force in the gelation process. The L-lysine organogel also showed a significant dendritic effect. The thermally reversible sol-gel transition temperature (T_{gel}) increased with increasing generation numbers (Figure 3). This positive dendritic effect can potentially be utilized to tune the material properties of organic materials.

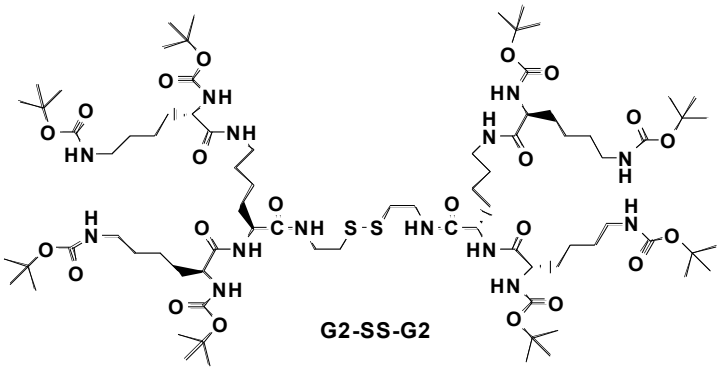


Figure 2. Dendrimer G2-SS-G2

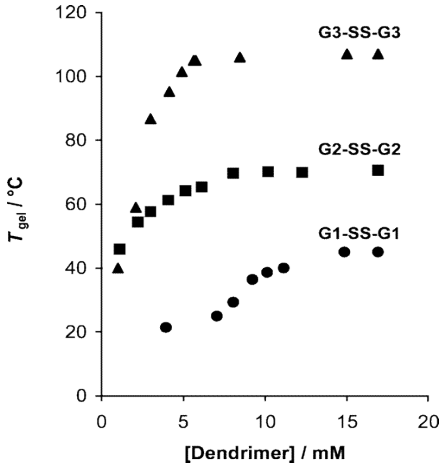


Figure 3. Effect of dendrimer concentration on the transition temperature (T_{gel}) in toluene

PEPTIDE DENDRIMERS AS PROTEIN MIMICS

Synthesis of artificial proteins has received much attention recently due to their potential applications in the study of protein integrations and development of protein-based materials.[12] Dendrimers possess many advantages including well-defined structure,[5] monodispersity, multivalency and ease of surface functionalization, which make them useful scaffolds for protein mimics.

Niwa and co-workers designed a three-generation, peptide-shelled dendrimer **1** (G3-PLGA) that adopts α-helical conformations in water (Figure 4).[13] Poly(L-glutamic acid) (PLGA) segments were covalently linked to the commercially available third-generation poly(amido amine) (PAMAM) dendrimer. A series of circular dichroism (CD) spectra were measured at pH values varying from 3 to 9 (Figure 5). A sharp decrease in helix content observed at pH 6.5-7 indicated transition of the peptide from helix to random coil.

Linear α-benzyl-L-glutamate-*N*-carboxy-anhydride (Pr-PLGA) polymer served as a control, and was

also studied by CD spectroscopy at varying pH values. As shown in Figure 5B, the helix content of the linear polymer was significantly lower than that of its dendritic counterpart, demonstrating the increase in helix stability in the dendrimer structure.

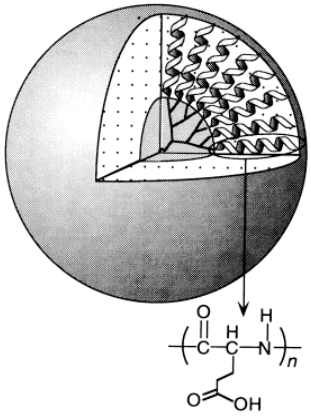


Figure 4. Spherical model of **1**

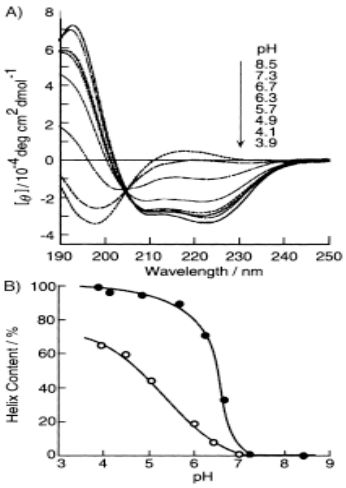


Figure 5. A. The CD spectra of **1** at various pH. B. The pH dependence of the helix content for **1**

The hydrodynamic radius of the dendrimer was also measured as a function of pH using Dynamic Light Scattering (DLS) (Figure 6). The steep increase of the dendrimer's diameter at pH 7 is in agreement with the conformational change from helix to random coil. Also, **1** (G3-PLGA) was shown to bind preferentially one enantiomer of α-amino acids having aromatic side chains. The R value (ratio of the amount of D isomer to L isomer bound by **G3-PLGA**) was found to be as high as 3, and was attributed to the hydrophobic interatctions between the PLGA peptide and the amino acid.

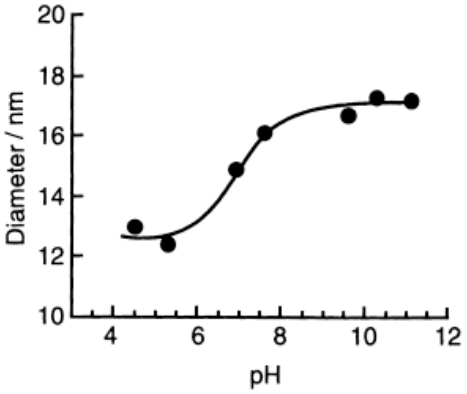


Figure 6. Hydrodynamic radius as a function of pH for **1** (G3-PLGA)

In efforts to mimic collagen, the main structural protein in mammals, Goodman and co-workers synthesized and studied a 162-residue dendrimer.[6] An *N*-Cbz-tris-(carboxyethoxymethyl)aminomethane (Z-TRIS[OH]$_3$) branching unit was functionalized with three peptide side chains and then three equivalents of the functionalized branching unit were coupled onto a trimesic acid core (Figure 7). The peptide chains were built from gly-pro-Nleu sequences, where Nleu represents *N*-isobutylglycine. Dendrimer **2** built from the gly-pro-Nleu sequence is shown in Figure 6. The CD spectrum showed that both the

Boc-α-Ala-TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$ dendron and the TMA[TRIS[(Gly-Pro-Nleu)$_6$ -OMe]$_3$]$_3$ dendrimer **2** adopted helical conformations at 8 ºC, but when the temperature was increased from 8 ºC to 22 ºC, only dendrimer **2** retained its triple helical structure. This indicates that the proximity of the peptide chains within the dendrimer enhances the side chain interactions and thus stabilizes the helical structure of the peptide within the dendrimer molecule. The melting transition temperature was independent of the concentration of the dendrimer, demonstrating that the observed peptide interactions are intramolecular.

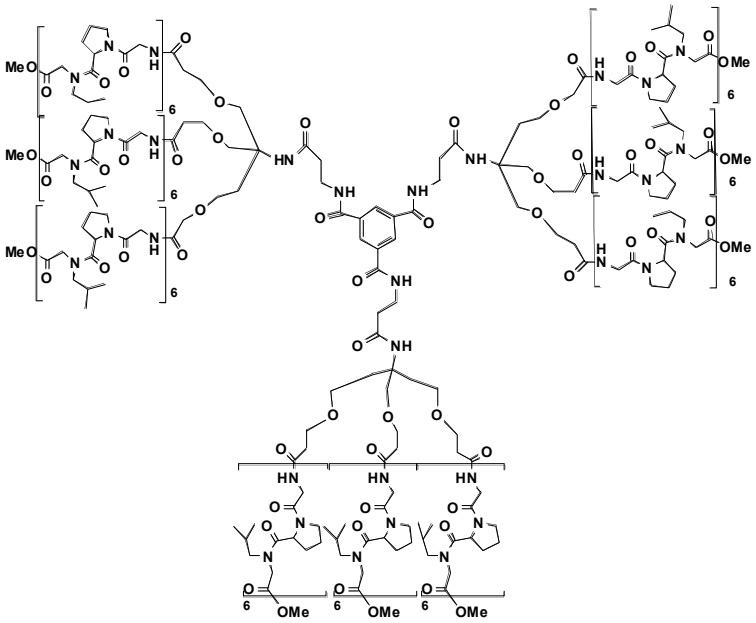


Figure 7. Dendrimer 2: TMA[β -Ala-TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$

PEPTIDE DENDRIMERS AS IMMUNOGENS

Peptide dendrimers have a wide variety of potential applications in biology, such as gene and drug delivery reagents, molecular inhibitors, and immunogens. The most common application of peptide dendrimers in the literature is based on multiple antigen peptides (MAPs).[5] Many examples of MAPs contain a branching unit and peripheral peptide chains; However, since they have no core, these MAPs are actually dendrons, not dendrimers. However, for convenience, they are still typically referred to as dendrimers. The advantages of using MAPs as immunogen scaffolds are simpler design and synthesis than peptide carriers, chemical unambiguity in generating site-specific antibodies, and versatility in immune responses.[5]

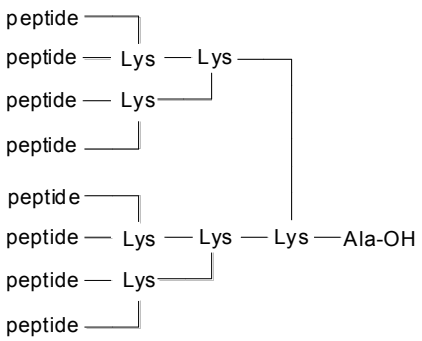


Figure 8. Synthetic MAP

In 1988, Tam and co-workers pioneered the use of lysine dendrimers as immunogens.[14] The structure of the MAP shown in Figure 8 has three components: a single amino acid that links the first lysine unit to the solid phase resin, an inner core of the third generation lysine dendrimers bearing eight free amine functional groups, and a surface layer of acylated peptides as synthetic antigens. One can see from the design that the bulk of the dendrimer is composed of the

surface-functionalized peptide antigens, which make up 80 % of the total mass of the molecule. Compared with protein-carried immunogens, a significant increase of immuogenicity of the dendritic MAP was observed in outbred rabbits.

In 1989, the same octavalent lysine dendrimer was applied by Tam et. al in vaccine engineering.[15] Two peptides, TN14 and LG15, representing two different epitopes of Hepatitis B Virus (HBV) envelope proteins were covalently linked to the lysine dendrimer. TN14 is a 14-residue peptide $(TKPTDGN)_2$ representing the a determinant of the S region; LG15 (LQDPRVRGLYFPAGG) represents a 15-residue peptide of the pre-S protein. Both homo-dimers and hetero-dimer were prepared as shown in Figure 9 and Figure 10, where black squares denote LG15 and hollow squares denote TN14.

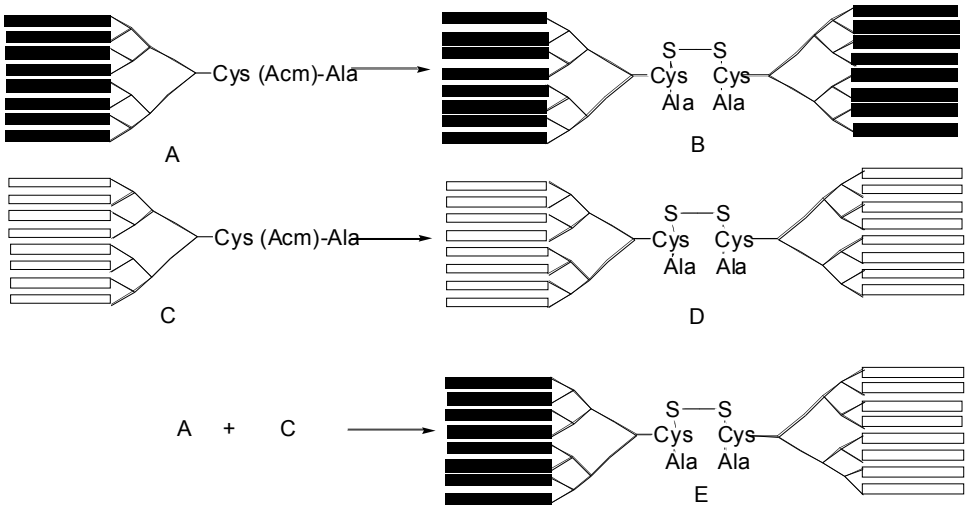


Figure 9. MAP of the homologous branching approach

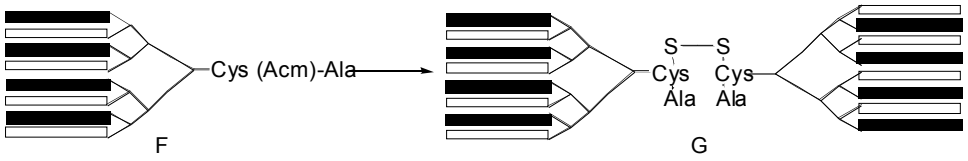


Figure 10. MAP of the heterologous branching approach

TN14 $(TKPTDGN)_2$

LG15 (LQDPRVRGLYFPAGG)

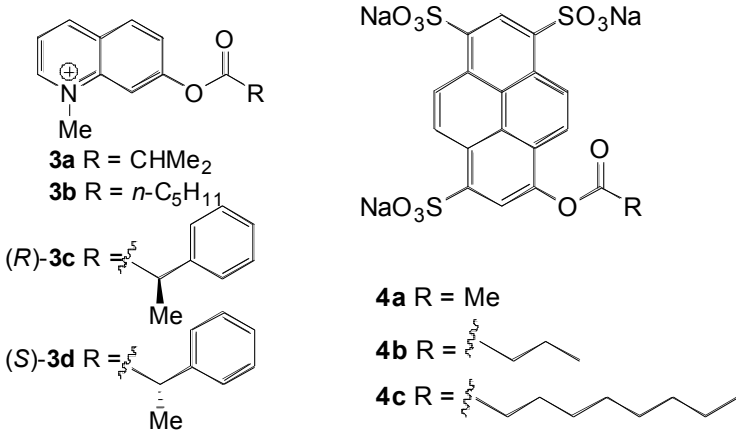
Immunization studies showed that dendrimer D containing only a TN14 monoepitope elicited nearly no antibody response, whereas dendrimer B containing only the LG15 elicited strong immune response. In addition, dendrimers E and G with diepitopes elicited the strongest responses. This indicates that the pre-S peptide determinant (LG15) acts as a T-helper cell by enhancing the immunogenicity of the S region and overcoming

the poor immune response of the S-protein monoepitope (14TN).

PEPTIDE DENDRIMERS AS CATALYSTS

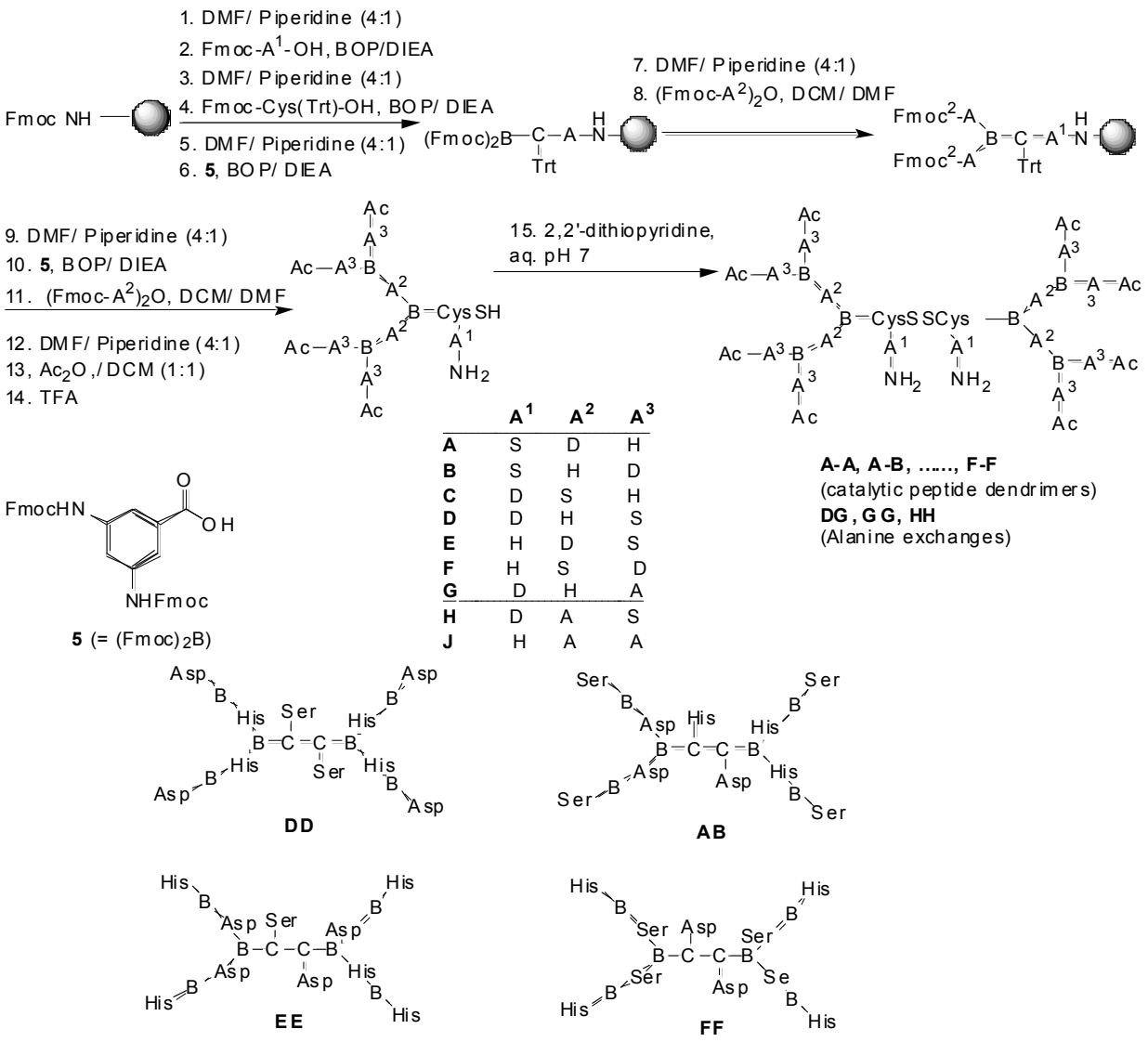
In addition to the applications discussed above, peptide dendrimers are also useful as novel macromolecular catalysts. Catalytic dendrimers were first synthesized in 1994 by Tomalia[16] and van Koten[17] by incorporating catalytic functionalities at either the dendrimer core or surface. The design of these dendrimer catalysts was inspired by the limitations of polymer-based catalysts such as catalyst leaching and lower catalyst loading due to polymer conformations that resulted in burying of active sites.[17] Dendrimers were found to be superior to polymers for these applications, since they can serve as homogeneous catalysts and can be removed from the solution by precipitation or filtration. Dendrimers also provide microenvironments for catalysis that are not present in linear polymers.

Catalytic peptide dendrimers, however, were not explored until 2003, when they were first synthesized by Reymond and co-workers.[19,20] They synthesized a series of peptide dendrimers having serine, aspartate and histidine residues at different positions of the dendrimer. Two of the synthetic dendrimers catalyzed the hydrolysis of 7-hydroxy-*N*-methyl quinolinium esters (3a-3d) in water and two others were found to catalyze the hydrolysis of hydropyrene-trisulfonate esters. Shown in Scheme 1 is the synthesis of the dendrimers. Monomers containing all six possible permutations of the three amino acids (ser, his and asp) were synthesized by solid phase peptide synthesis (SPPS) in 10-30 % overall yields, and dimerization of two monomers gave 21 different peptide dendrimers.



Dendrimers **DD** and **AB** showed strong catalytic activity at pH 6 for the hydrolysis of a variety of substrates, such as isobutyryl ester **3a**, hexanoyl ester **3b**, and 2-phenylpropionate ester **3c, 3d**, with K_m values of 0.1-0.55 mM and k_{cat}/ k_{uncat} values of 500-4000. More interestingly, dendrimer **DD** preferentially catalyzed hydrolysis of the *R* enantiomer of 2-phenylpropionate ester **3** with the relative rate ratio R = 2.8. The critical role of the histidine residues was confirmed by converting all aspartate residues into

Scheme 1. Solid Phase Synthesis of Peptide Dendrimer



	A^1	A^2	A^3
A	S	D	H
B	S	H	D
C	D	S	H
D	D	H	S
E	H	D	S
F	H	S	D
G	D	H	A
H	D	A	S
J	H	A	A

BOP: (benzotriazolyloxy)trisethylamino)phosphonium hexafluorophosphate; DCM: Dichloromethane
DIEA: diisopropyl-ethyl amine; Fmoc: 9-fluorenylmethyloxycarbonyl; Trt: Trityl

alanines to give modified dendrimer serine and a **JJ** that had similar catalytic activity. Competitive inhibition experiment with negatively charged 1,3,6,8-pyrene-tetrasulfonic acid demonstrated the contribution of electrostatic interactions in catalysis. The lower of K_m values of trisulfonate ester **4b** to **4c** with longer aliphatic chain length indicated the hydrophobic interactions between dendrimer and the substrate.

To further explore the contribution of dendrimer structures in catalysis, Reymond and co-workers synthesized peptide dendrimers having the same amino acids, but with higher generation numbers from G1 to G4.[21] Kinetic data showed a strong dendritic effect, as increasing the generation for G1 to G4 increased the catalytic efficiency per histidine side chain 4500 fold. Although the increase of generation introduces steric congestion between dendrons, the significant rate enhancement observed may result from increased

hydrophobic interactions between the dendrimer and the substrate.

CONCLUSION AND FUTURE DIRECTIONS

As a new class of biocompatible macromolecules, peptide dendrimers have found multiple applications including scaffolds for protein mimic, immunogens, synthetic vaccines, drug delivery reagents, and recently catalysts. While MAPs have been widely used in biochemistry and immunology, the catalytic peptide dendrimers are still at an early stage of development. Alternative peptide sequences and structural designs will be investigated in the future, and higher enantioselectivity in reaction catalysis may be possible. A combinatorial approach should be considered to screen a wider range of dendrimers.

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