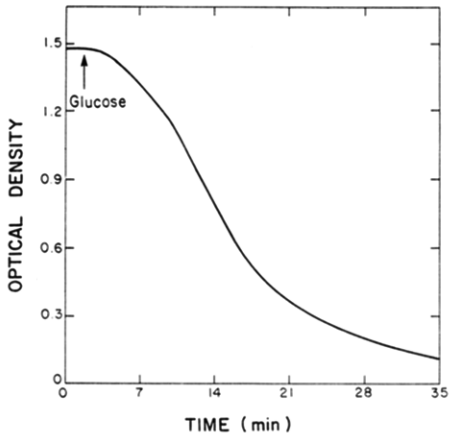

Figure 1. Optical density (400 nm) of an aqueous suspension of DLPC (2.4 mg/mL), PEAA (2.6 mg/mL), and GO (0.7 mg/mL) prior and subsequent to addition of glucose (1.3 mg/mL). Arrow marks time of glucose addition.

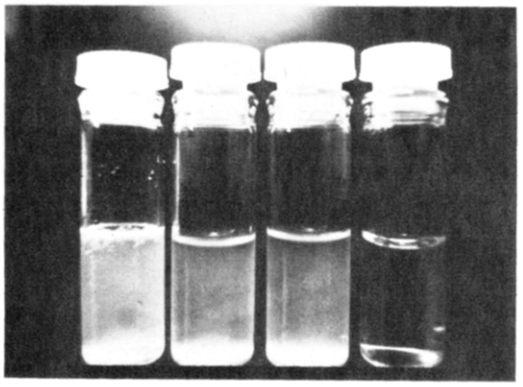

Figure 2. Clarification of DLPC/PEAA/GO suspension upon addition of glucose. From left: DLPC/GO; DLPC/GO + glucose; DLPC/PEAA/GO; DLPC/PEAA/GO + glucose.

causes no significant change in turbidity. Figure 1 is a plot of the optical density (at 400 nm) of an unbuffered DLPC/PEAA/GO (1/1/0.3) suspension vs. time prior and subsequent to addition of glucose[10] at a concentration of 1.3 mg/mL.[11] The optical density of the suspension is reduced rapidly following addition of glucose and reaches a final value less than 10% of the original after approximately 30 min.[12] Figure 2 demonstrates the clarity of the final suspension and presents the results of several control experiments in which no changes in turbidity were anticipated or observed; in particular, the addition of glucose to *polymer-free* DLPC/GO suspensions caused no loss in the turbidity of the suspension.

A full interpretation of these results must await thorough characterization of the aggregates present in the final, acidic suspension; however, disruption of DLPC vesicle membranes in acidic PEAA solutions has been demonstrated previously[4] and is believed to result from lipid solubilization in the compact, hydrophobic polymer coil.[13,14] In the present work, acidificiation occurs via enzymic generation of gluconic acid, with the result that the suspension displays a remarkable sensitivity to low concentrations of glucose.

These results are of interest from several points of view. First, we have shown previously that disruption of vesicle membranes by PEAA is accompanied by rapid, quantitative release of vesicle contents;[4] thus one can imagine therapeutic applications of the present work in self-regulated insulin delivery or diagnostic uses in monitoring of glucose concentrations in physiologic fluids. Second, the behavior of this system bears crude but real analogy to the behavior of hormonal second messenger systems[15] in that the process of interest is mediated not by the added organic solute (glucose) directly but by a second substance (H^+) generated in a specific way via enzymic catalysis. In this crude analogy, glucose plays the role of hormone and H^+ that of second messenger. Finally, this idea is quite general, in that many hydrolytic and oxidative enzymes are known to generate H^+ from a variety of substrates.

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(8) Poly(α-ethylacrylic acid) was prepared by radical polymerization of α-ethylacrylic acid as described in ref 4. The sample used in this work had an inherent viscosity (0.2% in DMF, 35 °C) of 0.19 dL/g.
(9) Glucose oxidase (from *Aspergillus Niger*, 40 000 units/g) was used as received from Sigma Chemical Co.
(10) D-Glucose (Gold Label) was used as received from Aldrich Chemical Co. The suspension was saturated with O_2 over a period of 10 min prior to addition of glucose.
(11) Normal concentrations of glucose in plasma of nondiabetic humans are in the range 0.7–1.4 mg/mL. Lehninger, A. L. *Biochemistry*: Worth: New York, 1975; p 831.
(12) In the experiment described by Figure 1, the initial pH was 7.4, and the final pH was 6.1. On the basis of the titration data in ref 1 and 2, the degree of ionization of PEAA should be reduced from ca. 50% to ca. 10% over the course of the reaction. We have not determined the extent to which DLPC modifies the titration behavior of PEAA.
(13) A similar mechanism has been suggested to account for pH-dependent solubilization of simple hydrocarbons[13a] or aromatic hydrocarbons[13b] in aqueous solutions of poly(methacrylic acid): (a) Liquori, A. M.; Barone, G.; Crescenzi, V.; Quadrifoglio, F.; Vitagliano, V. J., *Makromol. Chem.* **1966**, *1*, 291. (b) Chen, T.; Thomas, J. K. *J. Polym. Sci., Polym. Chem. Ed.* **1979**, *17*, 1103.
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Dendritic Macromolecules:[1] Synthesis of Starburst Dendrimers

A supreme challenge to synthetic chemists has been to design and construct molecular prototypes that mimic key functions in evolutionary chemistry. Host–guest com-

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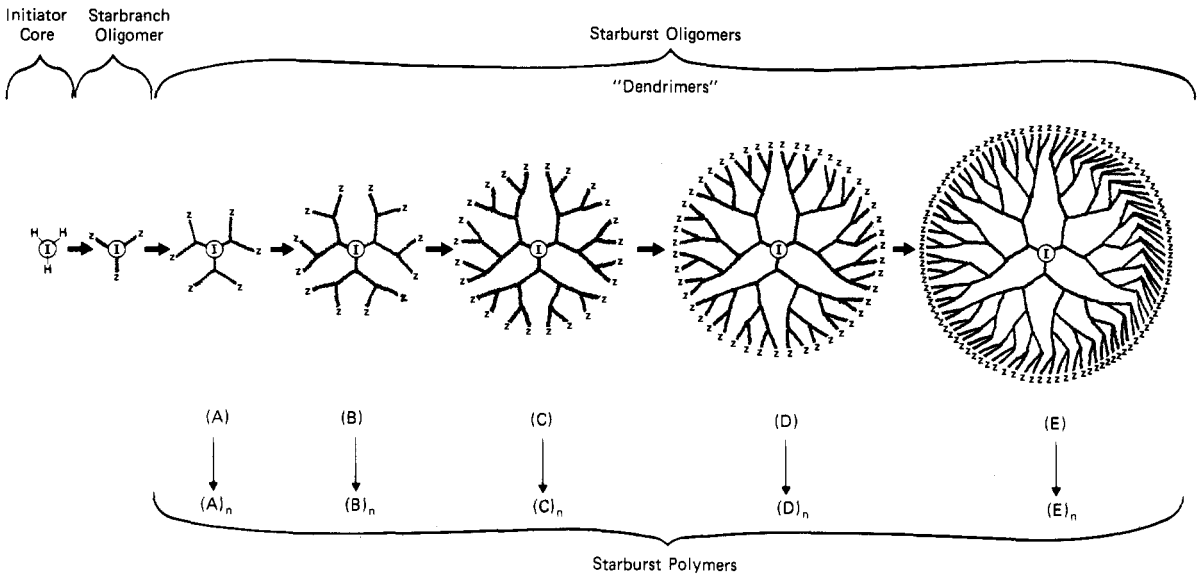

Figure 1. Starburst oligomers and their polymers.

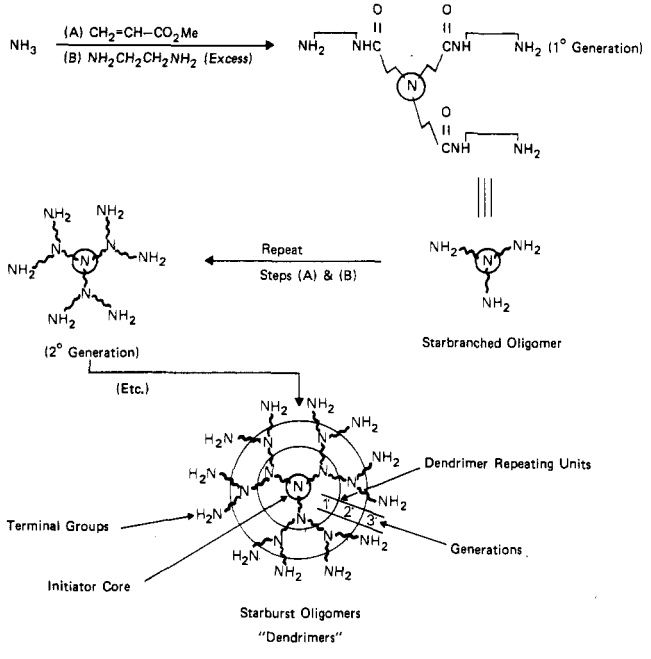

Figure 2. Dendrimer synthesis.

plexation,[2a,b,3,4] molecular self-organization,[5,6] and steric control of microenvironment[7,8] are just a few of the efforts that have focused on such an objective. A principal theme underlying many of these activities is to attain control over molecular size, shape, and disposition of organic moieties. In this quest, we elected to examine molecular architecture possessing regular dendritic branching with radial symmetry, which has coincidently received substantial theoretical interest recently.[8-11] Preliminary accounts by Newkome et al.[12] and Denkewalter et al.[13] have described the preparation of related cascade molecules. This work prompted us to report our synthetic progress, spanning the past 5 years, on such idealized molecular prototypes as shown in Figure 1.

We refer to these radially symmetrical molecules as possessing "starburst topology".[13] Entry into this topological regime involves construction of concentric, dendritic tiers around an initiator core. One proceeds first through a star-branched oligomer intermediate and then to the "starburst topology" by introducing both multiplicity and self-replication (within each tier) in a geometrically progressive fashion. These highly functionalized, dendritic molecules have been coined "dendrimers"[14] in deference to their branched (Greek = dendritic; tree-like) as well as their oligomeric nature.

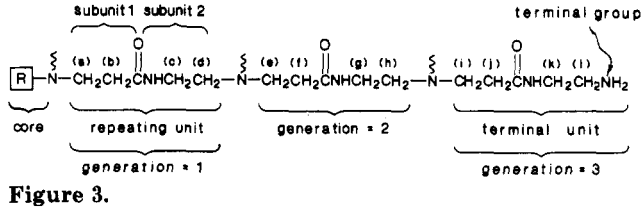

Figure 3.

The successful synthesis and characterization of these entities has allowed us to demonstrate the *controlled occupation of microenvironment as a function of size, shape, and disposition of desired organic functionality.* Subsequent covalent bridging of these dendrimers produces a new class of toplogical polymers, with size- and shape-controlled domains, which are referred to as "starburst polymers", i.e. $(A)_n$, $(B)_n$, $(C)_n$, etc.

Dendrimers are unique in that they possess three distinguishing architectural features, namely, (a) an initiator core, (b) interior layers (generations, G) composed of repeating units, radially attached to the initiator core, and (c) an exterior or surface of terminal functionality attached to the outermost generation. The initiator core type (i.e., NH_3 vs. alkyleneamines) can affect the dendrimer shape, producing, e.g., spheroids or ellipsoid-shaped molecules, respectively.[15] Advancement of generations determines the dimensions ($\simeq$10–20 Å per generation for this prototype) whereas the surface chemistry can be varied widely, based on moieties that may be derived from esters or amines.

Synthesis of the present poly(amido amine) (poly(β-alanines)) requires a two-step process, involving (I) exhaustive Michael addition to a suitable amine initiator core with methyl acrylate and (II) exhaustive amidation of the resulting esters with large excesses of ethylenediamine. The most fundamental prototype examined involved the synthon described in Figure 2, using ammonia as the initiator core. Construction of the interior generations involves proceeding from initiator core $\rightarrow$ star-branched oligomer (ester terminated, G = 0.5; amine terminated, G = 1.0) $\rightarrow$ starburst dendrimers (G = 1.5, 2.0, 2.5, ...). Interruption of this sequence at subunit 1 leads to the triester that is formed (98%) by addition of methyl acrylate to methanolic ammonia solution (see Figure 3). Addition

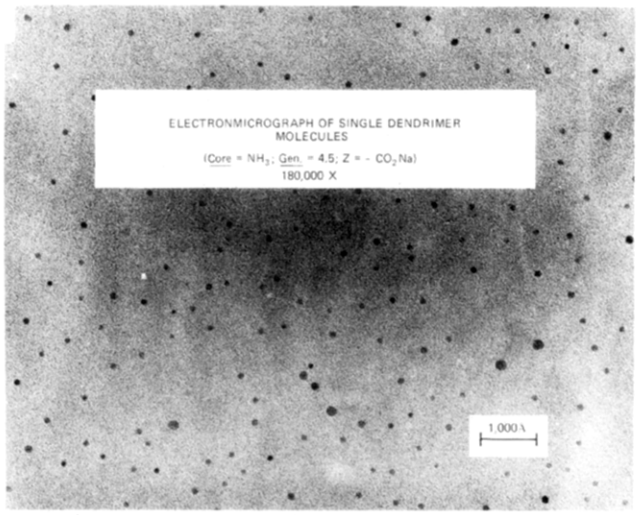

Figure 4. Electron micrograph of single dendrimer molecules.

of the triester to an 82-fold molar excess of ethylenediamine (EDA) (MeOH, 55 h/25 °C) with high-vacuum removal (50 °C/0.1 mm) of volatiles introduces subunit 2, yielding the amine-terminated star-branched oligomer (G = 1.0; 99%) (honey-colored oil): ^{13}C NMR δ 49.6 (a), 33.8 (b), 42.6 (c), 40.8 (d), 175.9 (*C*O); the mass spectrum showed the parent ion peak at *m/e* 359. Tribenzamide formed under Schotten–Bauman conditions (97%); mp 178–181 °C; IR (KBr) 3280, 3040, 2920, 2850, 1640, 1605, 15505, 1490, 1440 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.6 (3 H, amide), 8.1 (3 H, amide), 7.9 (6 H, ortho), 7.5 (9 H, meta and para), 3.4 (12 H, H-3,4), 2.8 (6 H, H-1), 2.4 (6 H, H-2). Anal. Calcd for $C_{36}H_{47}N_7O$: C, 64.4; H, 6.75; N, 14.60. Found: C, 64.4; H, 6.81; N, 14.36. Addition of triamine to a methanolic solution of methyl acrylate (10% molar excess) gives the hexaester (A) (G = 1.5); liquid (98%). Size exclusion chromatography indicates ≃99% single component. Reaction (72 h/25 °C) of hexaester with excess EDA (326 molar excess) in methanol gives (G = 2.0) a viscous yellow syrup (97%); ^{13}C NMR δ 49.6 (a), 33.7 (b), 37.8 (c), 52.2 (d), 50.1 (e), 33.8 (f), 42.7 (g), 40.8 (h), (*outer* CO) 176.1, (*inner* CO) 175.7. Low-angle laser light scattering (LALLS) gave MW = 1005 (theory 1043). Dendrimers B–E, where Z = CO_2Me or NH_2, were obtained in 91–99% yield as viscous syrups or glasses.[16,17] It was discovered that coordination of terminal functionality with group 1 metal ions provided surprisingly good electron scattering and contrast, thus allowing direct observation of single dendrimer molecules by CTEM techniques. Group 1 metal ions (Na^+, K^+, Cs^+, or Rb^+) were attached to dendrimer surfaces by hydrolysis of ester-terminated dendrimers with stoichiometric amounts of group 1 metal hydroxides. Very dilute solutions of dendrimer (D) (48X-CO_2Na) were placed on a beryllium grid (~1.5-mm-diameter puddle) and evaporated to dryness. A concentration continuum developed from the perimeter of the puddle, where single spheroidal dendrimer molecules were observed (see Figure 4), to a second region where a variety of aggregation types were noted and finally to the center, where a very dendritic-like microcrystalline growth occurred in the area of highest concentration.[17] A particle size count of the spheroids (Figure 4) revealed that they were highly monodispersed, with 86% of the particles measuring 88 ± 10 Å out of a total count of 47 particles. Measurements taken from two-dimensional collapsed/extended CPK models (as shown in Figure 1 for G = 4.5 (D)) predicted a dendrimer diameter of ≃78 Å. *It appears we may be observing individual dendrimer molecules resulting from the accumulative electron scattering effect of the sodium ion clusters on the dendrimer surface.* The larger particles are presumed to be aggregates of the fundamental dendrimer particles derived either from covalent bridging or from electrostatic attraction.

Such molecular arrays that possess both convergent and divergent binding sites[3c,d] have important implications in the synthesis of certain biocatalytic mimics. Examination and development of this area are in progress.

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Registry No. (Methyl acrylate)·(EDA) (copolymer), 26937-01-9; NH_3, 7664-41-7.

Supplementary Material Available: Experimental and analytical methods for structural validation (18 pages). Ordering information is given on any current masthead page.

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(14) We acknowledge A. J. Vogel for coining this very appropriate term.
(15) Preliminary observation of ellipsoid or rod-shaped molecules has been made and will be reported on later.
(16) Elemental analyses of key intermediates were in good to excellent agreement with theory.
(17) Synthesis details, electron microscopy experiments, related dendrimer defect chemistry, and analytical structure verifications are presented in the Supplementary Material.

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