CHEMISTRY

Dendrimers: Dream Molecules Approach Real Applications

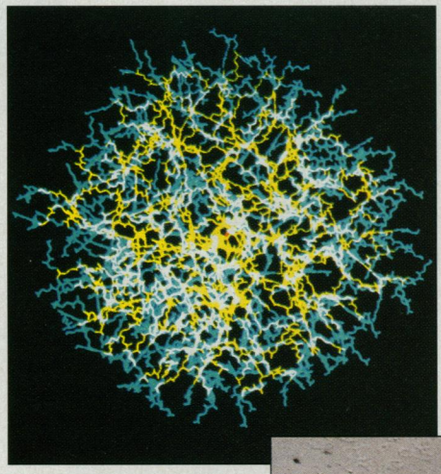
Dendrimers have a dreamy look. These synthetic molecules resemble billowing, three-dimensional snowflakes. And since their discovery in the late 1970s, dendrimers have been the stuff on which dreams are made. Because of their size—up to about 150 angstroms—and the way their branching architecture yields a dense surface surrounding a relatively hollow core, scientists have proposed using them as everything from artificial cells to drug carriers. But the materials science community as a whole has been slow to believe in these dreams, partly because dendrimer-makers couldn't prove their creations had the precise, regular structure needed for these applications.

Now it appears some dendrimer dreams may be coming true. New spectroscopic techniques developed in the last several years, such as electrospray mass spectroscopy, have given scientists a much better idea of the regularity of the dendrimers' branches, and that has started to convince the doubters. So researchers now are developing dendrimers as recyclable chemical catalysts and others that act as DNA-transfer vectors for gene therapy. And in this month's issue of *Chemistry of Materials*, researchers report taking initial steps toward making electrically conductive dendrimers; they hope such molecules may demonstrate unusual qualities, such as superconductivity.

"It's not just a pipe dream anymore," says University of South Florida chemistry professor George Newkome, a leading dendrimer researcher. "We are starting to control things outside and inside the dendrimers to make them functional." Others caution, however, that it will take more than control for dendrimers to become a commercial success—especially in the area of gene therapy—and many obstacles remain to be overcome before dendrimers leave the world of dreams altogether.

Dendrimers, also known as arborols or fractal polymers, can be made from virtually any material. They start from a core molecule with at least three chemically reactive arms. To these arms, chemists attach multiple branches. Because these new branches end with the same reactive groups as the original three arms, the process can be repeated many times. As the density of branches increases, the outermost branches arrange themselves in the form of a sphere surrounding a lower density core (*Science*, 29 March 1991, p. 1562).

Now chemists have begun to decorate that sphere with a variety of other molecules, making, among other things, highly selective chemical catalysts. The most selective catalysts are known as homogeneous catalysts, so called because they are dissolved in the same solution that contains the reactants upon which they act. (Heterogeneous catalysts, in contrast, can be solids, while the reactants are liquid or gas.) Homogeneous catalysts owe their selectivity to their small size (typically 10 to 15 Å) and well defined structure; larger catalytic molecules can have defects or odd shapes that reduce their selectivity. But these tiny catalysts are difficult to isolate and recycle when the reactions are complete.



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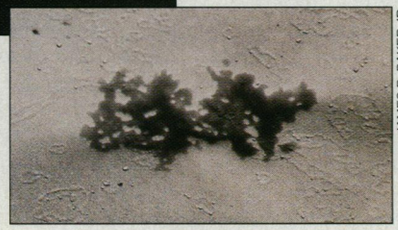

JAMES R. BAKER JR.

Branching into therapy. Branching molecules called dendrimers *(above)* are being tested as gene-therapy vectors because of their ability to bind DNA *(right)* and shuttle it into cells.

And chemical manufacturers would dearly like to get them back, as they typically contain precious metals such as platinum.

Dendrimers might offer a way around this manufacturing impasse. In a paper in the 15 December 1994 issue of *Nature*, a group of Dutch researchers showed they could stud the surface of a silicon-based dendrimer with nickel-containing catalytic groups. The dendrimer measured only 15 Å in size. But the molecule can also be grown larger than 20 Å and thus be recoverable through a filter, says one of the group's leaders, Gerard van Koten, a chemistry professor at Utrecht University.

The new dendrimer catalysts neatly transform a pair of chemical reactants into a chlorinated alkane, which serves as a starting material in the production of insecticides. The reaction produced "exclusively the [product] we were looking for," says van Koten, noting there were no unwanted by-products. The reason dendrimers work so well is that "all the catalytic groups are accessible at the surface of the molecule," making them easily accessible to the reactants, he says. Adds Newkome: "I don't think there's any doubt that these [dendrimers] will be used as commercial catalysts."

Dendrimers are also branching out into molecular medicine, as gene therapy vectors. This area is currently dominated by genetically engineered versions of viruses known as retroviruses. These viruses are very effective at delivering genes to cell nuclei. But they are small, meaning that they can only fit a small amount of genetic material in their cargo bays.

Researchers have tried to navigate their way around this problem by synthesizing gene vectors out of balls of fatty molecules called liposomes, which theoretically should be able to carry much larger genes. Although it's not entirely understood how synthetic vectors deliver genes to cells, researchers do know they bind to DNA because their positive charge attracts the negatively charged DNA molecule. The strength of that charge is related to the size of the synthetic molecule, so dendrimer researchers suspected their ability to precisely control the size and charge of their molecules would strengthen DNA binding and thus improve transfection efficiency over that of liposomes.

Their hopes received a boost when University of California, San Francisco, researchers Jean Hensler and Frank Szoka published an article in the September 1993 issue of *Bioconjugate Chemistry* showing that dendrimers made from a hydrocarbon-based polymer called PAMAM successfully carried the genes for reporter proteins such as luciferase into a cell. (These proteins have no clinical value, but their activity in the cell is used to measure the success of gene-therapy vehicles.) And these dendrimer-DNA complexes were "slightly more efficient than the best liposomes" in inserting the luciferase gene into cells in culture and getting it expressed, says Szoka.

Even though the experiment worked, careful analysis by Szoka's group showed many of their dendrimers to have defects, such as broken branches. So James Baker Jr. of the University of Michigan Medical School in Ann Arbor has been using PAMAMs made by Donald Tomalia of the Michigan Molecular Institute in Midland, who is credited with making the first well characterized dendrimers. And in unpub-

lished work, Baker says he has been able to use them to make dendrimer-DNA complexes that have now successfully transferred genes to more than 30 different types of cells with an efficiency up to 1000 times higher than that found by Szoka.

Those results have aroused some interest from gene therapy researchers. "I believe dendrimers have a serious future as vectors for gene therapy," says Claude Nicolau, a professor of medicine at Harvard Medical School, who has spent years working on liposome vectors. Thus far, however, dendrimers have not made the jump from success in the petri dish to success in live animals, he adds. Indeed, it's far from certain that dendrimers will ever make that jump, says James Wilson, director of the Institute for Human Gene Therapy at the University of Pennsylvania in Philadelphia. Polylysines, another DNA-binding synthetic vector, also looked good at transfecting cells in culture, he notes, but they failed miserably when it came to tests in live animals.

Researchers may be more successful in attempts to use dendrimers to move electrons around rather than genes. Larry Miller and his colleagues at the University of Minnesota, for example, have added electronically active groups called imides to the outer branches of PAMAM dendrimers in hopes of engineering a new class of 3D conducting polymers that may have unique electrical properties. The conducting polymers that are currently available, such as polyacetylene, are linear molecules. But in recent years researchers have found that 3D conducting molecules have unique properties not found in their linear counterparts. The soccerball-shaped carbon molecules known as fullerenes, for example, superconduct (conduct electricity without resistance) at a temperature higher than that of any other molecular conductor.

To make dendrimers with the right properties for this job, Miller's group starts with spherical PAMAM dendrimers that have 192 branches to which they attach pancake-shaped imide groups. Miller suspects this will allow electrons to jump from imide to imide around the surface of the sphere (although to demonstrate this he must first fashion the dendrimers into a larger material).

Others think the work holds promise. Formulating the spheres "sounds like a significant advance in terms of controlling the surface chemistry" of dendrimers, according to Martin Bryce, professor of chemistry at the University of Durham in England, who is working toward the same goal but using different conductive molecules as dendrimer add-ons. However, "you shouldn't get too excited about applications yet," he cautions. But a little excitement, the dendrimer-makers feel, is finally warranted.

–Robert F. Service

IMMUNOLOGY

The T Cell Receptor Begins To Reveal Its Many Facets

It's not hard to understand why the T cell receptor is an object of intense interest among immunologists. By recognizing and binding foreign antigens, the receptor (actually a collection of several proteins located in the outer membrane of the T cells of the immune system) helps trigger many of the immune responses that the body needs to fight off viruses and other pathogens. Just as important, it plays a key role in normal T cell development. As researchers delve deeper into how the T cell receptor operates, however, they are finding that its biochemical behavior is not at all what they expected.

Immunologists long assumed that the receptor was a simple on-off switch for the T cell: Bind enough of an appropriate antigen (protein fragment), and the receptor would activate the cell's full range of responses; otherwise nothing would happen. But recent work from several research teams, one of which reports its current results on page 515, shows that it doesn't work that way. Depending on the exact chemical nature of the antigen, the receptor can produce a spectrum of cellular responses, ranging from complete activation to inhibition. "People thought of the receptor as relatively simple and dumb," says Alessandro Sette of the Cytel Corporation in La Jolla, whose group is one of those doing the research. But he adds, it "may be a lot smarter than you think."

"People thought of the receptor as relatively simple and dumb, [but it] may be a lot smarter than you think."

—Alessandro Sette

The "intelligence" of the T cell receptor may underlie such fundamental processes as immunologic memory and "thymic education," in which the thymus gland selects out T cells that recognize and respond to foreign antigens while eliminating those likely to attack the body's own tissues. What's more, it may have implications for preventing rejection of organ transplants and treating autoimmune diseases such as multiple sclerosis and rheumatoid arthritis, which apparently occur because immune cells forget their thymic lessons and mistakenly damage normal tissue.

The current findings are an outgrowth of the growing understanding of immune cell activation that has occurred in the 12 years since the T cell receptor was discovered. Work from many labs has shown that T cells don't recognize antigens by themselves. Instead, they need help from a collaborator, the antigen-presenting cell (APC), which breaks protein antigens into short fragments or peptides. Individual peptides, usually consisting of eight to 14 amino acids, are displayed on the surface of the APC in association with one of the proteins encoded by the genes of the major histocompatibility complex (MHC). What the T cell receptor recognizes and binds to—its "ligand" in the jargon usually used—is a peptide bound to the MHC protein.

Early evidence that the T cell receptor behaves much more subtly than a simple light switch in responding to its ligands came about 4 years ago from Brian Evavold and Paul Allen of Washington University School of Medicine in St. Louis (*Science*, 31 May 1991, p. 1308). They were investigating how changes in the composition of an antigenic peptide affect T cell activation. When they replaced one glutamic acid residue with an aspartic acid residue, they found that the resulting peptide caused the target T cells to display only part of the activation response: The cells released a substance called interleukin-4, which is an immune-system modulator, but they did not undergo the rapid cell division that normally accompanies activation. "In immunology that was a surprising result," says immunologist Ronald Germain of the National Institute of Allergy and Infectious Diseases (NIAID). "The key thing was that it showed that the response could be qualitatively, not just quantitatively, different, depending on the peptide."

Since then, Allen's team has provided additional examples of altered peptides that elicit partial T cell responses, such as stimulating cell-killing activities without causing the release of modulators like interleukin-4 or cell proliferation. These peptides also induce anergy in the T cells, making them unresponsive to stimulation by the unaltered parent peptides. This finding suggests that the T cell receptor's many functions include sending signals that shut the T cell off.

Indeed, this work has been complemented by studies in which researchers, including Sette and Germain, have made altered T cell ligands that block T cell responses to normal peptides without eliciting