Cover Story

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CARBOHYDRATE VACCINES

Novel chemical and enzymatic oligosaccharide synthesis techniques could lead to a new generation of carbohydrate-based vaccine agents

STU BORMAN, C&EN WASHINGTON

Vaccines--agents that stimulate antibodies or immune cells to fight disease--have generally been made from weakened or killed pathogens or from immunogenic proteins, glycoproteins, or polysaccharides obtained from microorganisms. The carbohydrate-based agents--glycoproteins and polysaccharides--can be difficult to isolate from their natural sources, though, and the natural isolates can have heterogeneity and contamination problems.

An alternative would be to identify antigenic carbohydrates and then synthesize them in the laboratory. Oligosaccharide synthesis is so difficult that this hasn't been practical. However, that situation has begun to change in the past few years, as researchers have developed novel chemical and enzymatic techniques for constructing oligosaccharides more easily and conveniently.

These synthetic techniques are making it possible to envision a new generation of carbohydrate-based vaccines. Indeed, the first commercial vaccine made from a synthetic carbohydrate was approved last November in Cuba and is now part of that country's national vaccination program. Many other such vaccines are being developed and tested, and the number of synthetic-carbohydrate vaccines could increase significantly in coming years.

PLAN AHEAD Optimer Pharmaceuticals chemists Steve Sucheck (left) and Chang Hsing Liang discuss synthesis.
OPTIMER PHARMACEUTICALS PHOTO

Synthetic-carbohydrate vaccines have a couple of potential advantages over those based on carbohydrates from natural sources. Naturally derived carbohydrates are heterogeneous mixtures and may include small amounts of natural impurities and contaminants. In contrast, synthetic carbohydrates can be produced as homogeneous single compounds in a controlled manner with little or no batch-to-batch variability. In addition, medicinal chemistry techniques can potentially be used to derivatize and modify synthetic carbohydrates to make vaccines that are more immunogenic than those based on natural carbohydrates.

Some researchers also point out that carbohydrates are cheaper to produce synthetically, and this can help bring down the price of vaccines, which are often needed most by people in developing countries who cannot afford expensive medications. Other scientists disagree, contending that isolating carbohydrate-based immunogens from fermented microorganisms is not particularly difficult or expensive. Nevertheless, interest in and development of synthetic-carbohydrate vaccines seems to be a full-steam-ahead enterprise.

SYNTHESIS. Recently developed carbohydrate synthesis techniques are making it possible to create novel immunogenic carbohydrates "in sufficient quantities and pure form," says chemistry professor Peter H. Seeberger of the Swiss Federal Institute of Technology, Zurich. "There's more and more success in using these methods to make defined carbohydrate antigens." The advent of synthetic carbohydrate vaccines is "a triumph of tremendous advances in oligosaccharide synthesis," says chemistry professor Samuel J. Danishefsky of Memorial Sloan-Kettering Cancer Center (MSKCC) and Columbia University, both in New York City. "Without the years people have put into synthetic organic chemistry and carbohydrate chemistry, you wouldn't be able to make the kinds of investigational vaccines you can today."

Biotech companies are now leveraging such synthetic techniques for the creation of new candidate vaccines. For example, Optimer Pharmaceuticals, in San Diego, has been using a programmable, one-pot, solution-phase synthesis technique developed by Scripps Research Institute chemistry professor Chi-Huey Wong and coworkers, which the company calls OPopS, to create testable amounts of carbohydrate cancer antigens identified by Danishefsky's group. In the technique, a computer program is used to select presynthesized carbohydrate building blocks that are designed to combine sequentially in a single reaction vessel.

Meanwhile, Ancora Pharmaceuticals, Cambridge, Mass., has been using an automated solid-phase synthetic technology developed by Seeberger and coworkers to produce carbohydrate antigens for evaluation as possible vaccines for malaria, human immunodeficiency virus (HIV), tuberculosis, and bacterial infection.

Small companies like Optimer and Ancora aren't the only firms interested in carbohydrate-based vaccines. "Major vaccine manufacturers are expected to move in this direction soon," Seeberger says.

WON'T HURT A BIT
Quimi-Hib, the first approved vaccine based on a synthetic carbohydrate, is about to be administered to this baby in a Cuban clinic. The vaccine is a preventive against *Haemophilus influenzae* type b.
CENTER FOR GENETIC ENGINEERING & BIOTECHNOLOGY, HAVANA, CUBA

***HAEMOPHILUS INFLUENZAE* TYPE B.** Technical details about the synthesis, testing, and development of the first approved human vaccine based on a synthetic carbohydrate were reported last month [*Science*, **305**, 522 (2004)]. The vaccine prevents *Haemophilus influenzae* type b (Hib), a bacterium that causes pneumonia and meningitis, primarily in infants and young children. The vaccine was developed by Vicente Verez-Bencomo and Violeta Fernández-Santana in the department of chemistry at the University of Havana, Cuba; Eugenio Hardy at the Center for Genetic Engineering & Biotechnology, Havana; Maria E. Toledo at the Institute for Tropical Medicine Pedro Kouri, Havana; René Roy in the department of chemistry and biochemistry at the University of Quebec, Montreal; and coworkers.
Synthetic-carbohydrate Hib vaccine candidates were made and tested by two other groups. But "this is the first time that a synthetic carbohydrate component, after being attached to a protein carrier, has become a commercial vaccine," Roy says. "It's commercial in Cuba right now, and I've been in touch with a company that seems to be interested in commercializing it in the U.S."

To synthesize the carbohydrate component--a polyribosylribitol phosphate--the team used a one-pot solution-phase oligomerization process devised by reader in organic chemistry Andrei V. Nikolaev and professor of molecular parasitology M. A. J. (Mike) Ferguson at the University of Dundee, in Scotland.

Synthesizing the carbohydrate-based agent permits higher quality-control standards, compared to the use of naturally derived agents, Roy says. "It would not be surprising to me if more and more vaccines based on synthetic carbohydrates are developed in the near future."

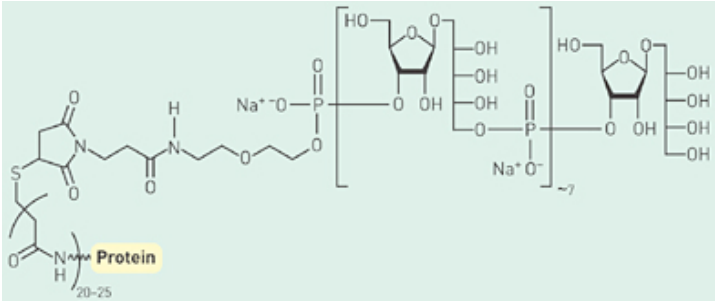
PAST IS PROLOGUE. There may well be a great future ahead for carbohydrate vaccines, but they also have a venerable past. The history of the field goes back at least to the 1920s and '30s, when Rockefeller University scientists Karl Landsteiner, Oswald T. Avery, and Walter Goebel showed that nonimmunogenic carbohydrates from bacteria could be converted to immunogens by attaching them covalently to proteins. For example, Avery synthesized the repeat unit of a polysaccharide from the capsule (shell) of pathogenic *Pneumococcus*, bound it to a protein, induced antibodies, and showed that the antibodies conveyed protection from exposure to the encapsulated microorganism. Around 1980, two research teams, working independently, developed semisynthetic carbohydrate-based Hib vaccines. One team consisted of microbiologist Porter W. Anderson Jr. of the University of Rochester and the late pediatric infectious disease specialist David H. Smith; the other included John B. Robbins, laboratory chief, and Rachel Schneerson, section head, of the National Institute of Child Health & Human Development (NICHD), Bethesda, Md.

Robbins says the two research teams "injected the capsule of Hib into children and adults and found that the capsule alone induced antibodies and conferred protection in older children and adults, but not in infants." This restriction was overcome by chemically combining the polysaccharide with a protein. Hib conjugates are now marketed commercially by several companies and used for routine immunization of infants throughout the world.

According to Roy, the synthetic carbohydrate-based Hib vaccine developed by him and his Cuban collaborators exhibits safety and efficacy that are about equivalent to those of the current commercial Hib vaccines, which contain naturally derived carbohydrates. Roy believes the synthetic-carbohydrate alternative has at least two potential advantages: its low production cost and its consistent composition. Robbins disagrees with Roy's first point: "The cost of nonsynthetic vaccines is not a problem. That's a myth."

Also around 1980, principal research officer Harold Jennings at the National Research Council of Canada's Institute of Biological Sciences, Ottawa, and coworkers, including Roy, used a conjugation approach to develop and commercialize a semisynthetic carbohydrate-based vaccine that protects infants against bacterial (group C) meningitis, the most common form of meningitis in young children. Once again, they didn't synthesize the carbohydrate per se. Instead, they derived polysialic acid from meningitis bacterial capsules and used chemical synthesis to combine it with carrier protein, making it immunogenic. This synthesis is now the basis for a commercial meningitis vaccine.

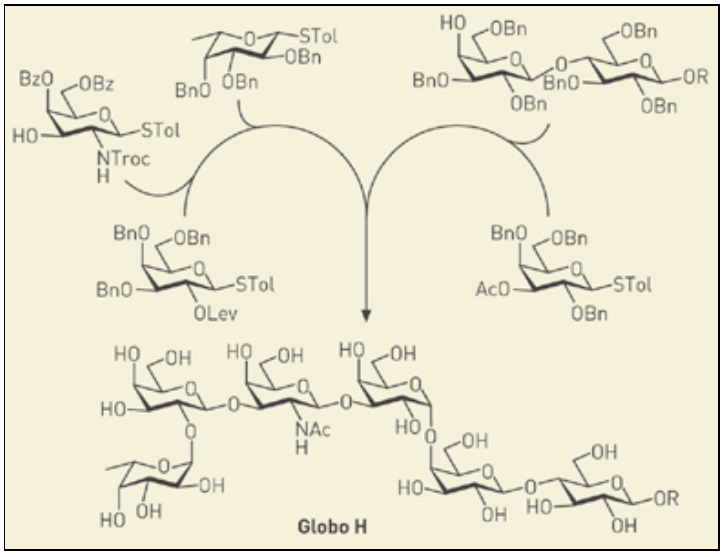
Although such classic vaccines are based on naturally derived carbohydrates, synthetic-carbohydrate conjugates are the vaccine candidates of the future, Robbins says. "When you synthesize a carbohydrate, you can make a very precise construct, and you can control the variables of chain length and chain density because you have a pure product"--in contrast to naturally derived carbohydrates, which are "mixtures of molecules."



SUGARY POLYMER The approved Cuban vaccine Quimi-Hib is a conjugate between a polyribosylribitol phosphate oligosaccharide and a carrier protein.

MALARIA. The Cuba-approved Hib vaccine could soon be joined by synthetic-carbohydrate vaccines for a number of other diseases. For instance, a malaria vaccine based on a synthesized carbohydrate antigen is currently in active development. Each year, hundreds of millions of people worldwide catch malaria, symptoms of which include chills, fever, sweating, anemia, adverse brain and kidney effects, and sometimes death. A few years ago, Louis Schofield and coworkers at the Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia, purified and characterized malaria toxin from the parasite that causes the disease. Seeberger and coworkers used their solid-phase automated synthesis technology to synthesize an oligosaccharide similar to the toxin's carbohydrate moiety, and the resulting vaccine was found to work in mice.

"To make a synthetic version of the malaria molecule back in 1995, it took five people about two-and-one-half years, and they got infinitesimally small amounts of material," Ancora Pharmaceuticals President John Pena says. "Now, with Seeberger's technology, we can assemble it inside of a few weeks and have milligram-to-gram amounts of material." Ancora has licensed the vaccine and is currently developing it. It's now in late preclinical tests, and Ancora hopes to begin clinical trials next year. A major pharmaceutical company that declines to be identified is showing interest in the vaccine as well.



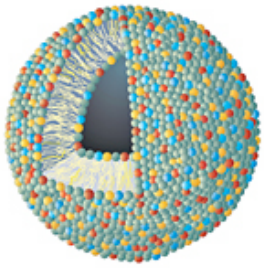
CONVERGENCE Wong's programmable one-pot-synthesis approach is used to link four monosaccharides and one disaccharide to make Globo H hexasaccharide. This is then linked to a protein carrier using the R group to make a candidate breast cancer vaccine. Ac = acetyl, Bn = benzyl, Bz = benzoyl, Lev = levulinoyl, STol = thiotoluyl, Troc = 2,2,2-trichloroethoxycarbonyl.

CANCER. Cancer is another major disease in the sights of synthetic-carbohydrate researchers. "No one is arguing that vaccines will knock out large tumors," Danishefsky says. "However, many cancer patients go through a stage that seems to be free of disease, such as after surgery, radiation, or chemotherapy. There's a high proclivity for recidivism of the cancers. But when they come back, they're not necessarily full-blown tumors. Those are what

we hope antibodies will block, although that hasn't been established yet." Danishefsky and coworkers have identified and synthesized a carbohydrate called Globo H that acts as an antigen on some human breast cancers. A Phase I clinical trial of a Globo H-protein conjugate plus an adjuvant (immune activator) was recently carried out at MSKCC.

The Globo H vaccine has been licensed to Optimer Pharmaceuticals. The company is using an OPopS-based synthetic method to make a sufficient amount for additional Phase I trials next year and for Phase II trials later. A major pharmaceutical company is interested in possibly codeveloping the Globo H vaccine.

Optimer researchers are looking for additional cancer-specific carbohydrate structures that could potentially be used in vaccines. "Our plan is to prepare glycoarrays, screen them with serum from cancer patients to identify undiscovered epitopes [recognition sites] on tumor-specific antigens, synthesize carbohydrates similar to those antigens, and then conjugate them to carrier proteins to create cancer vaccines," says Yoshi Ichikawa, Optimer senior director of chemistry. Such antigens can also potentially be used as targets for tumor diagnostic agents, he notes.



FATTY SPHERE
Carbohydrate vaccines are often formulated as liposomes (spherical lipid assemblies). In the above schematic of a bilamellar liposome, different-colored small spheres are antigen and adjuvant molecules and squiggly lines are lipid chains.
COURTESY OF SARAH LEHMAN, BIOMIRA

Danishefsky and coworkers are currently working on a new unimolecular multivalent vaccine. "It's a single glycopeptide that contains five antigens," Danishefsky explains. "It induces antibodies against all five." He believes the multivalent construct could be more effective against tumor cells containing a range of different antigens. It's already been licensed to Optimer and a major pharmaceutical company for further development.

Another carbohydrate-based cancer vaccine called GMK, which contains the sugar ganglioside GM2, is being developed by Progenics Pharmaceuticals, Tarrytown, N.Y. GMK vaccination induces anti-GM2 antibodies that target melanoma cells in a very specific manner. The vaccine is currently in Phase III trials for malignant melanoma.

And Biomira, in Edmonton, Alberta, has just completed a Phase III trial of Theratope, a carbohydrate-based vaccine for breast cancer. "Theratope didn't perform very well in the overall patient population, but it appears to benefit a subpopulation of patients who were already taking hormonal therapy for cancer before entering the trial," says Biomira Senior Director of Chemistry R. Rao Koganty. The vaccine is a conjugate of a carbohydrate tumor antigen called STn with a carrier protein.

Biomira also has other carbohydrate-based vaccines in preclinical development, such as the glycopeptide vaccine BGLP40, a small synthetic segment of a mucin glycoprotein that is expressed on cancer cells and includes multiple carbohydrate and peptide epitopes.

With the exception of Theratope, all of Biomira's vaccines are liposome formulations, in which antigens and adjuvants are incorporated into spherical lipid assemblies. Liposomes can carry multiple antigens and adjuvants and can enhance immune responses.

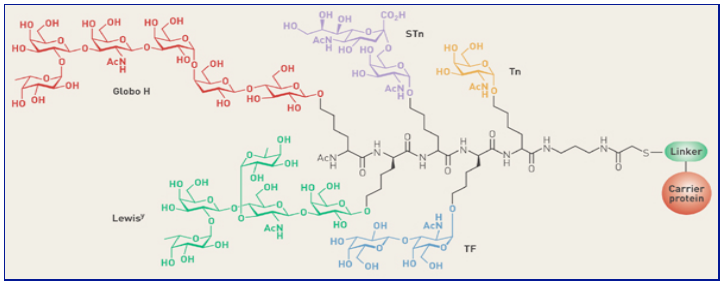
The carbohydrate portions of all of Biomira's carbohydrate-based vaccines are chemically synthesized. "We developed synthetic processes for large-scale production in commercial quantities," Koganty says. "We strongly feel that chemical synthesis is the best way to make carbohydrate vaccines, as natural carbohydrates and glycopeptides are too scarce and too difficult to isolate. Carbohydrate vaccines are coming up in a big way now, and I think they'll do very well in the next few years."

Koganty notes that carbohydrates are also important as adjuvants. Researchers have recently begun to consider adjuvants "not only as immune stimulants necessary to initiate responses to antigens, but also as molecules that interact with receptors of the immune system and trigger responses of their own that are being increasingly seen as therapeutic," he says.

AIDS. Attempts to develop all sorts of AIDS vaccines have so far been unsuccessful, but work continues, and carbohydrate-based vaccines are part of such efforts. For example, Danishefsky and coworkers synthesized the carbohydrate part of a vaccine designed to induce antibodies against gp120, the viral envelope glycoprotein of HIV. A human antibody called 2G12 "binds the carbohydrate part of gp120, and people who make that antibody can survive HIV infection for a long time," Danishefsky says. His group synthesized the carbohydrate moiety of gp120, the idea being that such an agent might induce 2G12-type antibodies that could protect against HIV infection. A major pharmaceutical company is currently working on a gp120-based agent, he says. "It's been conjugated to a carrier, and I believe the vaccine will soon go into [animal trials in] guinea pigs and then monkeys."

Wong and coworkers synthesized gp120-like oligosaccharides and screened arrays of the oligosaccharides against 2G12 to find optimal gp120-type antigens. Vaccine candidates based on the antigens so identified are currently being tested in mice at Scripps.

Seeberger and coworkers also synthesized the carbohydrate portion of gp120, used carbohydrate arrays to define structures responsible for gp120–2G12 binding, and are currently testing a gp120-based vaccine candidate in animals. Seeberger notes that earlier nonsynthetic gp120 vaccines "failed in broad-based trials in Thailand because the carbohydrates were too diverse. The use of defined synthetic carbohydrates in conjugate vaccines should be better."



CLICK FOR FULL-SIZE IMAGE

FIVE-PART WEAPON Danishefsky and coworkers are investigating multiantigenic agents, such as the one shown here, as next-generation synthetic-carbohydrate vaccines. Globo H, STn, Tn, Lewis^y, and TF are tumor antigens; Ac = acetyl.
STACY KEDING, ELI LILLY & CO. (FORMERLY AT MSKCC)

OTHER CONDITIONS. Carbohydrate-based vaccines have also been developed, or are currently being developed, for other conditions, such as fungal, bacterial, and protozoan infections. For example, Robbins' NICHD group, in collaboration with Henry Shinefield and coworkers at the Kaiser Permanente Vaccine Study Center, Oakland, Calif., has made carbohydrate conjugate vaccines that protect against opportunistic staphylococcal infections. They have also made conjugated vaccines for *Shigella* and cholera, enteric pathogens with surface lipopolysaccharides.

A conjugate they created with Vi polysaccharide from *Salmonella typhi* "is the best typhoid vaccine that's ever been made," Robbins says. "It's the first that's effective in two- to five-year-olds, and it probably will be effective in infants." Unfortunately, "vaccines don't make much money," he adds. "The Vi conjugate vaccine is so revolutionary, and typhoid is such a common and serious disease around the world, but no manufacturer in the U.S. or Europe is interested in it."

Professor of carbohydrate chemistry David R. Bundle and coworkers at the University of Alberta, Edmonton, are working on a carbohydrate conjugate directed against *Candida albicans*, a yeast that causes candidiasis, a type of fungal infection that can affect the skin, mucous membranes, or bloodstream. A key glycoprotein in the *Candida* cell wall has a sugar that includes a ß-1,2-mannan component.

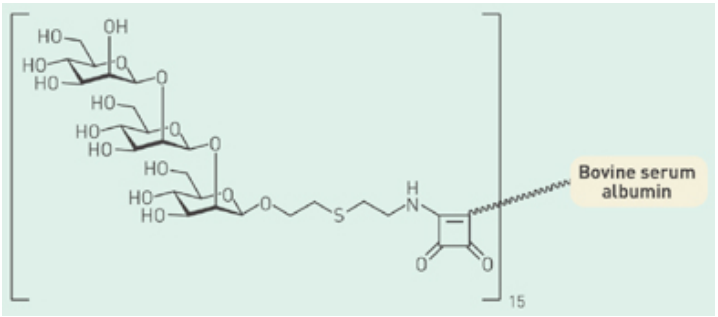
Pediatrics and microbiology professor Jim E. Cutler and coworkers at Louisiana State University Health Sciences Center, New Orleans, demonstrated that the ß-1,2-mannan is an important cell-wall antigen, raised antibodies against it, and showed that the antibodies could protect mice against *Candida* infection. Bundle's group and a team led by David Crich of the University of Illinois, Chicago, each synthesized the chemically challenging ß-1,2-mannans, helix structures with approximately three hexose sugars per turn. Bundle and coworkers then tethered some of these to proteins to make them immunogenic.

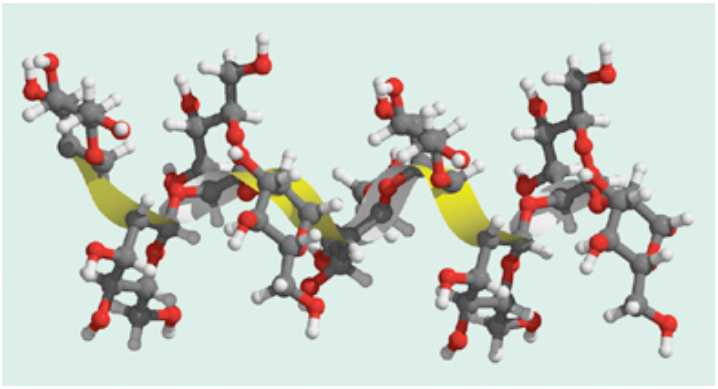
"Candidiasis is one of the most common hospital-acquired infections," Bundle explains. "We are currently testing a vaccine we synthesized to see if it can generate antibodies in animals before they are immunosuppressed. We will then challenge them with live *Candida* to see if they survive as a result of the antibodies." The vaccine has been licensed to TheraCarb, an Edmonton company that Bundle helped form, and codevelopment with either of two multinational drug companies is currently being considered.

Seeberger, a group at Ancora, and associate professor of chemistry Todd L. Lowary and coworkers at the University of Alberta are each independently pursuing synthetic-carbohydrate-based vaccines for tuberculosis. BCG (bacille Calmette-Guerin) is currently the only vaccine available for tuberculosis, but its efficacy is limited, and an effective alternative is being sought.

Seeberger and coworkers are also pursuing vaccines for the tropical diseases leprosy and leishmaniasis. A leishmaniasis carbohydrate they synthesized is currently being put into a liposome carrier system by Pevion Biotech, Bern, Switzerland, and the resulting vaccine candidate will be tested in an animal model.

The bottom line is that synthetic carbohydrates could prove to be a versatile basis for novel vaccines of many types. Ultimately, "synthetic vaccines against any unique disease-associated carbohydrate structures should be possible," Seeberger says.





UNDER STUDY **Bundle and coworkers synthesized a conjugate vaccine candidate for candidiasis (top). Ball-and-stick model of 9-mer version of the vaccine's ß-1,2-mannan carbohydrate moiety reveals its helical nature.**
UNIVERSITY OF ALBERTA

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