

Making Peptides At Large Scale

Efficient synthesis is helping to renew interest in the peptide drug market

[Ann M. Thayer](#)

Custom peptide manufacturers speak fondly of Fuzeon, a 36-amino acid HIV fusion inhibitor that has been on the market since 2003. It's not because the drug, developed by [Trimeris](#) and [Roche](#), is a blockbuster. In fact, many call it a flop because runaway sales of the high-priced treatment never materialized.

They like it because, to be effective, the daily dose of the poorly stable peptide is a hefty 180 mg. That dose makes Fuzeon one of a few peptide drugs to have been made in near-ton annual quantities. And beyond sheer volume, they like that it proved large-scale production of a long peptide is possible.

Fuzeon production broke ground in terms of equipment and process design. To couple that many amino acids takes a huge number of synthetic steps and massive amounts of raw materials. "All the amino acid suppliers had to gear up to supply that manufacturing effort, which brought the costs down for everyone," says Jim Hampton, executive vice president of business development at peptide supplier [AmbioPharm](#) in North Augusta, S.C.

Roche has stopped R&D on HIV and, in a restructuring move, put its [Roche Colorado](#) peptide manufacturing site up for sale. But its interest in peptide drugs seems to be growing, and like many big pharma firms it is collaborating with peptide discovery companies.

As a result, the peptide ingredient market is growing as well, and drug developers are asking custom manufacturers to make the newest generation of peptide drug candidates. Although these peptides are longer and more complex than earlier ones, technical improvements and economies of scale have made production faster and more cost-effective. By harnessing multiple synthetic and recombinant techniques, contract manufacturers are helping turn peptides into affordable and effective drugs.

"Peptides have been around for a long time, but only recently have come into prominence because research has led to the development of more robust molecules," says Ipshita Chakraborty, senior research analyst with the market research firm [Frost & Sullivan](#). "It's now possible to generate stable peptides, and the therapeutic peptide sector is venturing into new disease areas." About

60 peptide drugs are on the market, and nearly 10 times that are in preclinical and clinical development.

Today, 36 amino acids is about the average peptide length that suppliers are being asked to make. “The choice of production technology depends on the quantities that are needed and on potential challenges related to the peptide sequence,” says Johan Devenyns, general manager of Solvay’s [Peptisyntha](#) business.

Solution-phase chemistry is usually favored for peptides that are fewer than 15 amino acids long and for quantities greater than 100 kg. When it comes to complex or longer amino acid sequences, solid-phase chemical synthesis usually wins. Peptides, or what some call miniproteins, of 50 amino acids and longer can often be made by recombinant means.

“Peptide manufacturing is substantially more expensive than manufacturing small molecules, but it’s still often less expensive than using recombinant procedures for quantities under 50 to 100 kg,” says Rodney Lax, senior director of business development in North America at [PolyPeptide Group](#), which operates facilities in the U.S., Europe, and India. Making a peptide for clinical trials using recombinant methods can be up to an order of magnitude more expensive than chemical synthesis, he says.

A solid-phase process generally is the fastest and cheapest to develop, peptide suppliers tell C&EN. Solution-phase processes, despite the name, can actually require less solvent and also might avoid the need for chromatographic purification. Increasingly popular are hybrid approaches that combine solid- and solution-phase steps to make shorter fragments that are coupled to make bigger peptides.

Peptide active pharmaceutical ingredients (APIs) generally are made at the tens-of-kilograms scale, with about a dozen at the 100-kg-per-year level. Fifteen years ago, “a kilogram was just an unthinkable amount of peptide to have to make,” Hampton says. Today, leading manufacturers operate reaction vessels of up to a few thousand liters in size. “In peptide terms, this is huge,” Hampton says.

Solid-phase peptide synthesis involves sequentially coupling protected amino acids on a support. In the past five years, increased sourcing of raw materials from low-cost countries has significantly reduced costs for solid-phase synthesis, Hampton says. “Companies may derivatize them in the U.S. or Europe, but they are buying the basic amino acids from China.”

Founded in 2007, AmbioPharm has a business model of offering low-cost peptides made under current Good Manufacturing Practices (cGMP). In

addition to a South Carolina facility it acquired from the former UCB-Bioproducts, the company runs a cGMP plant in China. “The first step will be making generics because it will be a while before the world is ready for new chemical entities coming 100% out of China,” Hampton predicts.

Similarly, PolyPeptide is shifting some of its generic products to a facility it built near Mumbai, Lax says. “Most customers who are developing proprietary peptides, however, would prefer to keep them close to home in the U.S. or Europe.”

Generic peptide drugs are under the most price pressure but remain an important part of the market, suppliers say. “About half of peptide APIs are produced captively by pharma companies,” Devenyns says, which leaves the rest open to contract manufacturers. “The addressable market opportunity for peptide APIs is on the order of \$500 million per year,” he estimates. About 20 peptide drug candidates enter clinical trials each year, and anywhere from zero to three typically get approved.

Several peptide drugs will lose patent protection in the next five years, including Fuzeon and the large-volume anticoagulants Integrilin (eptifibatide) and Angiomax (bivalirudin). “The peptide market is still a relatively young one, but there are some very well established generics,” Frost’s Chakraborty says.

According to Hampton, many peptide manufacturers are opting to make bulk eptifibatide, a relatively simple cyclic heptapeptide, but only a few, such as AmbioPharm and [Teva Pharmaceutical Industries](#) are tackling the 20-amino acid bivalirudin. Both have completed drug master files. Teva became a significant competitor after buying the peptide drug firm CoGenesys in 2008.

Peptisyntha takes a “common fragment” approach to making some generic peptides. After identifying shared sequences among peptides, it can achieve economies of scale by pooling their syntheses. The company is adding a large-scale cGMP solid-phase peptide plant at its Brussels site, where it already has industrial-scale solution-phase capabilities. The company also practices solid-phase synthesis in Torrance, Calif.

For short peptides, Peptisyntha has developed a process to precipitate APIs at desired purity levels that avoids the need for chromatography. “We’ve also further developed our specific know-how in the area of lyophilization,” Devenyns adds, to improve productivity and control product morphology.

“Control over drug substance quality and morphology is becoming increasingly important to ascertain bioavailability and manage drug substance stability for new engineered-release peptide therapeutics,” says Mimoun Ayoub, Peptisyntha’s vice president of global business and strategic development.

And most opportunities will stem from developing new dosage forms and improving drug delivery.

Drug delivery firms are learning to formulate peptides for nasal, oral, and transdermal delivery. “If we find a drug delivery platform that is applicable to a wide range of peptides, it is really going to open doors and make peptide therapeutics of much greater interest,” Lax says.

Conjugation is also used to increase target specificity or extend half-lives. “Peptides are actually bridging the two worlds of small and large molecules,” says Stefan Stoffel, head of Lonza’s chemical manufacturing business. In its work, the Swiss company is seeing more antibody-drug conjugates and cell-penetrating peptides linked to cytotoxic drugs.

Requests for PEGylated peptides are on the rise as well. “We are being asked to make large quantities of these nowadays, as well as other conjugates,” PolyPeptide’s Lax says.

How a peptide will be formulated or delivered may have to be addressed during its actual synthesis. Peptide manufacturers also need to design their syntheses to make the more complex cyclic or constrained structures that developers are creating for better stability. These factors are frequently dealt with through backbone modifications or the insertion of nonnatural and functionalized amino acids in the sequence.

Producing peptides that are cyclic or contain nonnatural amino acids is “pretty sophisticated,” Devenyns says. Cyclic peptides can be formed, for example, by installing internal disulfide bridges across cysteine residues or by creating hydrocarbon links between functional groups.

Having these functionalities in place may require using customized amino acid derivatives, which some peptide producers also make. “We have in-house manufacturing of special complex building blocks and can design them to the exact purity that we need,” says Philip Ottiger, president of [Bachem Americas](#), part of Switzerland’s Bachem Group.

Developing a robust, large-scale manufacturing process can be challenging but is doable depending on the number of modifications, explains Jason Moss, a business development technical manager at Bachem, which has several production sites in the U.S. and Europe. “It can be a perfect storm of functional groups where any individual one won’t be that big a deal from a process chemistry perspective, but having the combination could require a lot of effort,” he says. Synthesis might call for multiple protecting groups and novel coupling and oxidation chemistries. First synthesized as linear, many peptides are subsequently folded stepwise to get the right connectivity and shape.

Subtle changes to the peptide structure can make a process scalable and economically feasible—or not. “A peptide could have one amino acid, for example, where a gram of it would take six months to make and cost \$100,000, but a slight one-carbon change makes it one that’s \$1,000 per kg,” Moss explains.

Peptide synthesis is highly sequence dependent, and method development and analytical characterization are critical as the process grows in complexity, suppliers point out. Synthetic routes must be broken down to determine, for example, where fragment solubility and racemization might be problems.

As the number of amino acids rises, so, too, does the number of potential impurities, many of which will be almost identical to the final product. “The dream is to be able to crystallize the desired peptide within the expected specifications without going through a purification by high-performance liquid chromatography,” Lonza’s Stoffel says. “We still have a long way to go in achieving this goal, and challenges remain in the purification stages to remove critical impurities that are close to the APIs.”

Further work is needed to minimize the loss of desired APIs during primary and secondary purifications, Stoffel says. “In the generic field or in the development of a second-generation product, the impurity profile match is critical from a regulatory point of view and is a challenge for the process development chemists.”

Scalability, rather than the underlying chemistry, is the main aspect of peptide synthesis that has changed over the past five years, contends Firuz Shakoory, director of sales at [American Peptide](#) in Sunnyvale, Calif. “Companies now are capable of making large quantities of drug substance in a single batch, and the size of purification columns has increased drastically,” he says.

American Peptide has added four purification suites in recent years, and it has plans for new solution- and solid-phase synthesis suites. It offers research-grade peptides from a facility in Sunnyvale and has cGMP production in Vista, Calif. Ito Life Sciences, part of Japan’s [Otsuka Chemical](#), has owned the firm since 2008.

As companies are scaling up to make larger volumes of peptides, the industry is consolidating into a core group of players, many with multiple sites and technologies. In 2007, PolyPeptide acquired Isochem’s NeoMPS business. [Lonza](#) bought UCB-Bioproducts in 2006. More recently, Diosynth became part of [Fujifilm](#) and Corden Pharma acquired [Genzyme Pharmaceuticals](#).

With sites in Belgium, Switzerland, China, and the Czech Republic, Lonza may be the only large-scale peptide supplier to offer both chemical synthesis and

recombinant production. According to Stoffel, “An estimated 8% of peptides use recombinant processes, which are mainly managed by pharma in-house production.”

Drug developer [Unigene Laboratories](#), for example, focuses on recombinant peptide manufacturing at its cGMP plant in Boonton, N.J. After expressing a peptide in *Escherichia coli*, its process includes an in vitro enzymatic amidation of the peptide tail to enhance stability, activity, and bioavailability. Its technology is being used to make development-stage products and has been licensed to Novartis.

The main advantage chemical synthesis has over recombinant methods lies in the ability to insert unusual amino acids into a sequence, Bachem’s Ottiger points out. This step is a prelude to creating peptides that can be specially cyclized or conjugated to other entities. Companies such as La Jolla, Calif.-based [Ambrx](#) are conducting research to create cell-based systems that can handle nonnatural amino acids.

Taking another approach, [Sutro Biopharma](#) in South San Francisco has developed biochemical protein synthesis in a cell-free system. Components of the system include a DNA template that encodes the desired peptide, a ribosomal extract as the synthesis machinery, amino acids, and an energy source, explains Chief Scientific Officer Trevor Hallam. Nonnatural amino acids can be incorporated by using transfer RNAs to deliver them at the appropriate point as the template is decoded.

“We can actually put a lot of chemical functionality into these, including reactive amino acids to let us do chemistry on the product,” Hallam says. The desired peptide is produced quickly, and proper folding can be promoted by controlling the chemical and physical environment. The product also can be easily separated, he adds.

Sutro’s goal is to make peptides that are inaccessible because they are not expressed by cells, cannot be isolated, or are insoluble. This year, the company entered a multiyear collaboration with Pfizer. “At the moment we are preparing to build clean rooms so we can actually work to cGMP. Our plan is to be setting up to produce clinical-trial materials by early next year,” Hallam says.

Although peptide manufacturers fall into two camps—chemical synthesis or recombinant production—most observers believe this dichotomy will disappear. But rather than one approach supplanting another, they are likely to complement each other or even merge. “The demarcation line between peptides and minproteins will disappear as well because there will be chemical

synthesis of much larger proteins and more recombinant manufacture of shorter peptides,” Lax says.

Chemical & Engineering News

ISSN 0009-2347

Copyright © 2011 American Chemical Society