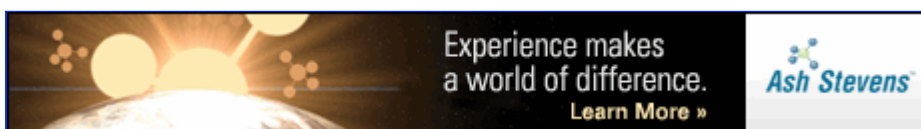


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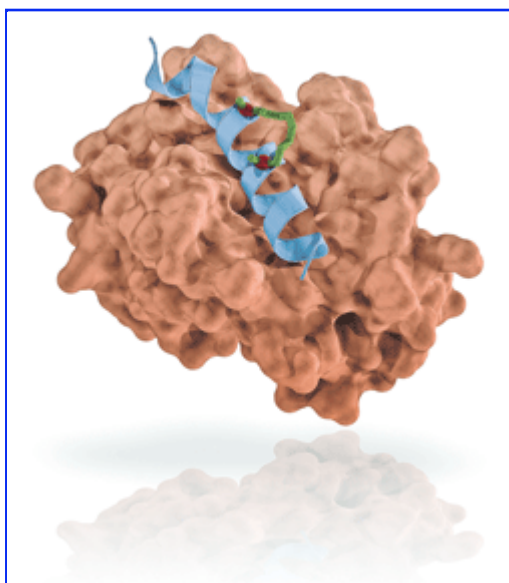
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Improving Peptides

Small firms develop better peptide drug candidates to expand this pharmaceutical class and attract big pharma partners

[Ann M. Thayer](#)



Aileron Therapeutics [View Enlarged Image](#)

LOCKED IN Added hydrocarbon links, or staples, can stabilize peptide conformations.

Peptide drug candidates, like long-distance runners, are undergoing training. Many more than in the past are trying to build the muscle and endurance to reach the finish line. And a few wins along the way are bringing out the fans.

“As the challenges traditionally associated with peptides, like stabilization and delivery, are addressed, and

more and more peptides progress through the clinic and are marketed, the space has matured and there's a lot more pharma industry interest," says Paul Watt, founder and chief executive officer of the Australian biopharmaceutical firm [Phylogica](#).

About 60 peptide drugs sold worldwide had combined sales approaching \$13 billion in 2010. Among these, the multiple sclerosis therapy Copaxone and the hormone-related products leuprolide, octreotide, and goserelin have annual sales of more than \$1 billion each.

Nearly half of approved peptide drugs are older generics, many of which are based on natural products. Newer products, such as [Eli Lilly & Co.'s](#) recombinant osteoporosis therapy Forteo and Amylin Pharmaceuticals and Lilly's synthesized diabetes drug Byetta, are fast approaching the \$1 billion milestone.

Peptides play central roles in bodily processes. The advantages they can offer as drugs—such as specificity, potency, and low toxicity—are well-known, but they have stumbled over practical hurdles such as poor stability, short half-life, and being readily digested by protein-eating enzymes in the body. Executives at small peptide discovery companies say they have found solutions that are making fans of some big pharmaceutical firms.

Indeed, more large companies are evaluating peptides as part of their drug development strategy, says Joseph A. Yanchik III, CEO of Cambridge, Mass.-based Aileron Therapeutics. "They understand that if you can unlock certain fundamental limitations of peptide therapeutics you might have the largest superclass of drugs that the industry has ever generated." As a result, small firms have attracted investors and large R&D deals. "Clearly the pharma partners are trying to accelerate this development," Yanchik adds.

About 140 peptide drug candidates are in clinical development. "There's been a decent uptick from the 1980s when about five new peptide molecules per year entered clinical studies," says Janice M. Reichert, senior research fellow at the [Tufts Center for the Study of Drug Development](#). "That went up to about 10 during the 1990s, and we're at about 17 per year now."

Reichert's figures come from a study she conducted for the [Peptide Therapeutics Foundation](#), which promotes peptide R&D. The nonprofit group's sponsors are [Amylin](#), [Ferring Pharmaceuticals](#), [Ipsen](#), [Pfizer's CovX](#) unit, and the peptide contract manufacturer [PolyPeptide Group](#).

Greater use of peptide drugs over the past two decades also reflects an acceptance of injected therapies by doctors and patients, Reichert points out. Depending on the disease indication, other delivery routes are being explored, including oral forms. Advances in delivery technology are expected to have a major impact on peptide drug development.

Approved peptide drugs and those in development fall into many therapeutic areas, with oncology, metabolic disorders, and cardiovascular disease on top. Most disease targets are extracellular, and these are dominated by G-protein-coupled receptors.

"Who would have ever thought that Gila monster saliva would be a good place to look for a type 2 diabetes drug?"

"There is a lot of research being done on cell-penetrating peptides, but this hasn't yet translated into a large number of molecules with intracellular targets," Reichert says. Only about 10% of clinical-stage peptide candidates are aiming for the hard-to-hit, albeit plentiful, intracellular targets.

Intracellular targets, among those often labeled as "undruggable," are challenging for two reasons, Watt explains. The first is the difficulty of simply getting the peptide into the cell. Adjusting the number of charged amino acids in a sequence, for example, is one way to make peptides pass through membranes. Then, once inside, peptide structures, such as those held together with disulfide bridges, may unravel in the reducing environment.

Phylogica, the result of a collaboration between Australia's [Telethon Institute for Child Health Research](#) and the [Fox Chase Cancer Center](#) in Philadelphia, works around these obstacles. "Many of our structures are very stable but are not constrained by disulfide bonds and therefore could remain stably folded even inside cells," Watt says. Small enough to cross cell walls, these rigid structures maintain the desired conformation for binding, but unlike linear peptides, they may resist being chewed up by protease enzymes.

Working with [Roche](#), Phylogica has identified peptides that can both travel into cells and deliver cargo. “Some of these have no structural resemblance to other well-characterized cell-penetrating peptides,” Watt points out.

Similarly, intracellular delivery is a goal of [KAI Pharmaceuticals](#) and [AngioChem](#), which specifically goes after blood-brain barrier targets. And last year, [Compugen](#) began offering an in silico process to predict cell-penetrating structures through which it has found 20 such peptides.

Founded in 2001, Phylogica has signed three partnerships with major pharmaceutical companies over the past 18 months, according to Chief Financial Officer Nick Woolf. The company has a deal worth up to \$100 million to find antibiotic peptides with [AstraZeneca](#)’s biologics unit, [MedImmune](#), and another on therapeutic peptide vaccines with Pfizer that could bring in \$135 million. It recently extended its collaboration with Roche to evaluate the potential for peptide delivery to the brain.

Relying on nature to provide a range of evolutionarily stable structures, Phylogica finds peptides that it calls Phylomers within bacterial protein fragments. To create libraries of these protein pieces, the sequenced genomes of diverse bacteria, primarily extremophiles, are fragmented. The genes are then expressed to create random, yet overlapping, peptide libraries.

The libraries can be screened for a desired phenotype, such as killing cancer cells, or for binding to a known target. Once an active Phylomer is found, “we are able to isolate the sequence that encoded the peptide and then order it as a synthetic peptide from a manufacturer,” Watt says. “We then test the isolated peptide for the activity we selected for in the first place.”

Although Phylogica has found peptides that bind with picomolar affinity, it can further optimize them via mutagenesis of the gene sequence and chemical modification. “Since every screen yields sets of structurally related peptides, we can fast-track the structure-activity lead optimization process,” Watt says. “The screens provide very powerful bioinformatic clues for where to focus mutagenesis without disrupting the peptide scaffold.”

Amino acids also can be replaced with functionalized or nonnatural ones to improve a peptide’s stability, activity, and half-life. The entire sequence can even be synthesized in reverse order—to form so-called retro-inverso peptides—using d-amino acids, which is a way to stabilize peptides and make them resistant to proteolysis, Watt says.

Ranging from 15 to 80 amino acids, but averaging about 30, Phylomers are small enough to be made cost-effectively by chemical synthesis, Watt says. At this size, they are amenable to noninjectable delivery methods. Phylogica’s experience so far has been with intranasal routes. Pointing to four marketed intranasal peptide drugs, Watt sees potential for success.

TOXIC ORIGINS				
Several successful peptide drugs have their roots in venoms				
DRUG	MOLECULE	ORIGIN	DISEASE	COMPANY
Aggrastat (trofiban)	Peptidomimetic	Saw-scaled viper	Angina/heart attack	Medicure
Byetta (exenatide)	Peptide	Gila monster	Type 2 diabetes	Amylin/Eli Lilly & Co.
Capoten (captopril)	Peptidomimetic	Brazilian lancehead snake	Hypertension	Bristol-Myers Squibb
Integrilin (eptifibatide)	Cyclic peptide	Southeastern pygmy rattlesnake	Ischemic stroke	Millennium Pharmaceuticals
Prnalt (ziconotide)	Miniprotein	Magician’s cone snail	Chronic pain	Elan/Azur Pharma

SOURCES: Bachem, Atheris Laboratories

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Other companies screen as well. With two marketed peptides, the diabetes drugs Symlin and Byetta, Amylin has amassed a library of peptide hormones. In collaboration with Lilly, it is developing nasal and transdermal forms of Byetta. An injectable long-acting version made by linking the peptide to [Alkermes](#)’ biopolymer microspheres faces regulatory delays in the U.S. but has been recommended for approval in Europe. In the obesity area, under a deal initially valued at up to \$1 billion, Amylin has been working with [Takeda Pharmaceutical](#), but in March the partners halted a clinical study on their lead project to investigate the possible neutralizing effect of antibodies on the tested drug combination.

[Affymax](#), once part of GlaxoSmithKline, also has been working with Takeda since 2006 to develop the anemia treatment peginesatide (once known as Hematide). Arising from Affymax' recombinant library, the synthetic peptide is PEGylated for stability and extended half-life. And Cambridge, Mass.-based [Dyax](#) screens for peptide candidates using phage display libraries, which it has licensed to many big pharma firms. In 2010, Dyax launched the angioedema peptide drug Kalbitor.

According to Tufts's Reichert, only about 10% of peptide drugs in development have been modified through means such as PEGylation, lipidation, radiolabeling, or conjugation to a small molecule, antibody, or protein. "But it seems to be a growing area as the technology advances," she says.

Phylogica recently agreed to work with [Pepscan Therapeutics](#) in the Netherlands to use that firm's technology for chemically linking peptides to scaffolds that will keep them in biologically active conformations. Under a European Union grant, Pepscan also is working with Copenhagen-based [Zealand Pharma](#) to create dual-action peptides. On its own, Zealand has licensed peptide drug candidates to Pfizer, [Helsinn Healthcare](#), and [Sanofi](#), although Pfizer returned its compound after deciding to exit cardiovascular R&D.



Protagonist Therapeutics [View Enlarged Image](#)

BUILDING BRIDGES Disulfide bonds maintain cyclic peptide structures.

On the list of successful synthetic peptide drugs are five originally found in venoms, which are highly complex mixtures of potent and selective bioactive compounds. Although it makes sense that venom-based peptides, such as Prialt, act as anesthetics, "who would have ever thought that Gila monster saliva would be a good place to look for a type 2 diabetes drug?" remarks Jason Moss, a business development technical manager at peptide supplier [Bachem Americas](#), referring to Byetta.

As the five products show, venom-derived peptides don't just act like venom but are useful against different disease targets. "These peptides have been designed evolutionarily to sit on receptors," Moss says about their potency and stability. To broaden access to them, Bachem has partnered with [Atheris Laboratories](#), a Swiss peptide and protein drug discovery firm. Bachem offers Atheris' venom libraries in microplates, under the name [Melusine](#), for screening.

Atheris can provide mixed or species-specific collections. Rather than supply pure venoms, which are too potent for cell assays and too complex to characterize, Atheris fractionates them using high-performance liquid chromatography, Moss explains. "It makes venoms a lot easier to screen for drug discovery and development."

When hits are found, Atheris can identify the active component and provide lead optimization for customers. "If a customer wants to develop a molecule, Bachem can prepare a research-grade batch synthetically to make sure it is identical and has the same activity," Moss says. After that, "we can provide material for preclinical and clinical development." Most peptides are made by chemical synthesis.

The fact that peptides can be manufactured at large scales under regulatory compliance has helped advance peptides as drugs themselves, rather than just as leads for small-molecule development, Moss suggests. "If you look at the history of peptide therapeutics from natural sources, many are 20 to 30 amino acids in size with one or two, sometimes three, disulfide bonds." Such structures are synthetically manageable, he says.

[Protagonist Therapeutics](#) focuses on disulfide-rich peptides, or DRPs, ranging from eight to 40 amino acids.

“DRPs address the major disadvantage of peptides, that of biological stability,” CEO Dinesh Patel says. Founded in Australia but headquartered in Redwood City, Calif., the company combines rational drug design and computational tools to identify DRPs, phage display libraries for expression and screening, and medicinal chemistry to optimize the structures and activity.

APPROVED PRODUCTS
 Numerous large sellers are among peptide-based drugs marketed in the past 10 years

GENERIC NAME	TRADE NAME	DISEASE TARGET	COMPANY	2010 SALES (\$ MILLIONS)
Teriparatide	Forteo	Osteoporosis	Eli Lilly & Co.	\$830
Exenatide	Byetta	Type 2 diabetes	Amylin/Lilly	710
Liraglutide	Victoza	Type 2 diabetes	Novo Nordisk	416
Lanreotide	Somatuline	Growth disorders	Ipsen	228
Pramlintide	Symlin	Diabetes	Amylin	92
Enfuvirtide	Fuzeon	HIV	Roche/Trimeris	88
Ziconotide	Prnalft	Pain	Elan/Azur Pharma	20*
Icatibant	Firazyr	Angioedema	Shire	11
Ecallantide	Kalbitor	Angioedema	Dyax	9
Tesamorexlin	Egrifta	Lipodystrophy	EMD Serono	na
Degarelix	Firmagon	Prostate cancer	Ferring	na
Mifamurtide	Mepact	Bone cancer	Takeda	na
Nesiritide	Natrecor	Heart failure	Johnson & Johnson	na

NOTE: Includes U.S. and non-U.S. approvals. * C&EN estimate. na = not available.

[View Enlarged Image](#)

Tapping into a diverse set of DRPs, Protagonist can identify leads against almost any kind of target in a de novo fashion, Patel claims. “If we have structural information on a target, then we will try to mimic what may be the best fit into the binding site,” he says. “If that is not available, we have created generic libraries that capture all different sizes and shapes offered by DRPs to screen against a target.”

Protagonist aims at targets not approached satisfactorily either by biologics or small molecules. “We are trying to grab the best of both worlds,” Patel says. “We want them to have the potency of biologics, but because of their engineered stability, we want them to have the pharmacokinetics and absorption, distribution, metabolism, and excretion properties of small molecules.”

Although many peptides fall apart in the stomach, “we are very, very confident in the oral stability of DRPs,” Patel says. In January, Protagonist entered a discovery and development collaboration with [Ironwood Pharmaceuticals](#). Ironwood has its own peptide drug, linaclotide, in Phase III clinical trials for irritable bowel syndrome. A cysteine-rich, 18-amino acid peptide with three disulfide bridges, it is stable enough to be taken orally.

Permeability is another requirement to make peptide drugs bioavailable. If small enough, they may be able to move unaided through the wall of the gastrointestinal tract. If not, permeability or absorption enhancers might be required. “In some instances, you might not want permeability if the targets are confined to the GI tract,” Patel explains. “In this case, you can convert low permeability into a huge advantage and have a very specific drug with an excellent safety profile.”

[Aileron Therapeutics](#) has developed a tool kit of stabilization technologies to retain peptides in the desired conformation. Initially, it used olefin metathesis to form hydrocarbon links, or staples, across functionalized amino acids in α -helices. The company has since licensed other chemistries and has developed its own technology.

Although α -helices are involved in many drug-target interactions, the company is not limited to that structure, Chief Scientific Officer Tomi K. Sawyer says. “Stapling chemistry comes with incorporating novel or nonnatural amino acids, but we like to leverage as much of nature’s original sequence as possible to take advantage of the high specificity peptides have for their targets, whether they are enzymes, receptors, or protein-protein interactions.”

Aileron’s targets are largely intracellular. The firm often starts with structural information on the target to design a peptide ligand, but it may also use diversity-based approaches for lead optimization, Sawyer says. Modifications of analogs can help fine-tune target specificity and cellular activity. “We are building into the same sequence both cell-penetrating ability and the pharmacophore to bind to a specific target, while being

metabolically stable. So you get all the features needed for a drug,” he says.

“The really powerful feature of stapled peptides is that they have extraordinarily good pharmacokinetic properties right from the start,” Sawyer adds. “We believe we can go neck and neck, certainly in terms of potency, with any small molecules.”

Such features are making pharma firms “recalibrate their thinking” about what’s possible with peptide drugs, Sawyer says. In 2010, Aileron signed a deal with Roche worth \$25 million up front and up to \$1.1 billion in potential milestones. The program is focused on undruggable targets in infectious and metabolic diseases and oncology.

In 2009, Aileron attracted investments from the corporate venture funds of [Lilly](#), [GSK](#), [Novartis](#), and [Roche](#). The Novartis and GSK funds have also invested in [Bicycle Therapeutics](#), a U.K.-based firm that creates chemically constrained peptides. And Lilly has backed Protagonist and [Sutro Biopharma](#), which is developing new peptide manufacturing methods.

Advances in manufacturing that make peptides more economically feasible have helped small companies, Yanchik suggests. “Technologies allow us to do things today that would have been very difficult even 10 years ago.” As a result, potential partners are expressing fewer concerns about whether new peptide drug candidates can be made.

Creating a helical structure, for example, takes at least 10 amino acids, and longer peptides may contain 40 or more. “There are pragmatic limits of how long you need to go to achieve target specificity versus how long you can go before your manufacturing cost of goods becomes a little bit high,” Sawyer says. “And you better make sure that the market you are looking at will be able to accommodate the more expensive, longer peptide.”

Inserting stapling modifications doesn’t compromise the scale or chemical yield, and the cyclization chemistry is very efficient, Sawyer claims. Aileron has developed manufacturing capabilities over its five years in operation.

“One of the pleasant surprises of the platform has been the ability to scale up the stapled peptide technology,” Yanchik says. Although the company can produce research-grade and higher quantities, it has gone to outside firms to test production under current good manufacturing practices.

Other peptide drug firms can do even more of their own manufacturing. Ferring is a Swiss peptide drug company that began by extracting peptide hormones from animal sources and modifying them. In the 1960s, it developed methods for chemical synthesis and now has several production facilities. With its acquisition of Bio-Technology General in 2005, it also gained recombinant manufacturing methods.

Similarly, [Unigene Laboratories](#) combines its recombinant manufacturing capabilities with drug delivery and formulation technologies to develop new peptide products. It has supplied salmon calcitonin to Novartis for osteoarthritis and osteoporosis products in development and has licensed its production technology to the drug company as well.

Unigene also developed Fortical, a nasal calcitonin product marketed by Upsher-Smith Laboratories. It has licensed an oral form to Tarsa Therapeutics, in which it holds a 25% stake. And, for many years it has worked with GSK under an agreement worth up to \$145 million to develop an oral formulation of a recombinantly produced parathyroid hormone analog for treating osteoporosis.

Those in the race indicate that discovery, development, and manufacturing challenges are increasingly being met. If there is any remaining skepticism to overcome, it is around regulatory approval. “Peptides do seem to take longer in regulatory review, averaging about two years,” Reichert says. Questions tend to fall in the chemistry, manufacturing, and control areas.

Nonetheless, proponents are optimistic about peptide drugs playing a bigger role, especially against undruggable targets. According to Protagonist’s Patel, “Just like biologics are now grasping a larger footprint at the expense of small molecules, similarly as we go forward I believe peptides are going to have a larger footprint at the expense of both biologics and small molecules.”

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