OVERVIEW

GAP Peptides, LLC (GAPP) is engaged in the development and commercialization of a novel method of peptide synthesis that shows potential for securing substantial cost savings when compared to traditionally accepted technologies. Early research substantiates that the method delivers high efficiency and makes positive strides toward reducing production time and direct costs associated with conventional synthesis methods.

Based on Group Assisted Purification (GAP) chemistry, GAP peptide synthesis (GAP-PS) facilitates many benefits the manufacturing industry seeks in a production method: high yields and high quality, fewer processing steps, less raw material and solvent consumption, and ultimately less waste.

Fresh research shows that GAP-PS provides quantifiable savings through synthetic efficiency in three key areas: 1) reduction or elimination of column chromatography; 2) reduction of raw materials (amino acids and coupling reagents) consumption; and 3) reduction of solvent usage, thus reducing reactor size and waste disposal costs.

Ripe for Change

Facing relentless economic pressures and margin erosion while striving to increase value delivery for their customers, manufacturers aggressively seek ways to reduce costs. Similarly, research and development sponsors bringing new peptide products and therapies to market seek lower cost options that simultaneously improve quality and reliability in peptide APIs and intermediaries. Leveraging a global strategy to gain cost savings, including lowering total cost on the manufacturing floor, is an option many organizations undertake. According to the FDA, in 2018 there were almost 270,000 registered facilities, more than 50% being overseas, that handled FDA products. However, a global strategy is not without risk. Recent US news reports point out numerous and broad failures at production facilities, particularly in India and China. While these countries dominate in the production of generic drugs, their reputations and public perceptions have been impacted by reports of inadequate quality controls, insufficient management oversight, and breakdowns in manufacturing practices needed to meet required specifications. These breaches create issues for developers and consumers of peptide-based products everywhere. Though the advantages of a global strategy are recognized, broader forces, including economic conditions, regulatory restrictions, raw material & resource availability, trade barriers, currency valuations, and geopolitical stability impact the overall cost and quality of peptide manufacturing. Innovation is crucial for finding solutions to the comprehensive challenges that drive up costs and vital for encouraging competition that results in better products for all.

Several recent FDA statements report that nine out of ten of America's prescriptions are for generics.[1] Furthermore, FDA statistics disclose that at least 80% of the active ingredients found in America's medicines come from abroad – primarily China and India. Rosemary Gibson, a national authority on health care, and critically acclaimed author of several books including "China RX: The Risks of America's Dependence on China for Medicine" recently stated, "*In five to 10 years we are at risk of losing our generic drug industry…China will undercut our own producers and drive them out of business…it's already happening.*" It is no secret that innovation and the presence of high-value manufacturing creates a boon for economic growth wherever that manufacturing occurs. With the peptide market growing, so grows the opportunity to drive productivity in the global economy which ultimately drives higher wages and better living standards for workers. From this perspective, innovation in peptide manufacturing has the potential to create benefits on many fronts globally.

The field of peptide manufacturing appears ripe for change. Modern, sustainable, innovative synthesis technologies that offer a positive step change in efficiency and economics could potentially galvanize and increase the manufacture of synthetic peptides. Aimed at tackling some of the direct costs of producing synthetic peptides, Group-Assisted Purification (GAP) peptide synthesis is a new approach to peptide synthesis that was developed by researchers at Texas Tech University. The process offers competitive advantages for companies seeking an alternative to outsourcing as a means of controlling production costs. Research and industry collaboration currently underway suggest that now may be an ideal time to consider the approach as an alternate tool for peptide manufacturers to include in their toolbox of synthesis options.

Innovating Peptide Chemistry

GAP chemistry uses specially designed chiral auxiliaries or protecting groups to control a wide variety of properties in the substrate molecule. These can include controlling stereochemistry, reactivity, and solubility, among other properties. By managing these key properties, syntheses can be more easily manipulated to optimize process performance. Perhaps the largest advantage of GAP chemistry lies in solubility control, where group assisted purification "*avoids traditional purification methods such as chromatography and/or recrystallization by introducing a well-functionalized protecting group,*" enabling selective precipitation of the GAP-protected substrate from the reaction mixture.[14] During the past few years, GAP chemistry has found success in small molecule synthesis, particularly chiral amine synthesis.

GAP peptide synthesis uses GAP chemistry to facilitate peptide assembly by using a 300 Da protecting group as a C-terminal anchor. The approach developed from the idea that the solubility control discovered in small molecule syntheses could be replicated in peptide synthesis by replacing the resin in SPPS with a uniquely designed GAP protecting group. In GAP-PS, reactions are run efficiently in solution, but the target peptide is selectively precipitated from the crude mixture, enabling a simple, filtration-based purification process. Research has shown that the idea has great potential in terms of scientific merit and commercial application.[2-4]

Improving Peptide Economics

Based on emerging research findings, the adoption of GAP peptide synthesis as an alternative approach for synthetic peptide production is a strong consideration. The method appears viable as a lower-cost option for manufacturers, thus enabling peptide-based products to be discovered, developed and synthesized faster and more economically than by using conventional methods. Emerging production efficiencies reveal benefits that appear practical and tangible: providing a needed catalyst to explore the potential of accepting the approach as a means of cutting manufacturing costs. The advantages delivered by GAP-PS include the following.

i.   Reduction or elimination of column chromatography

A key benefit from the use of GAP peptide synthesis is the high crude purity of the peptide generated. Practically all peptide API's are purified by chromatographic methods to one extent or another,

requiring expensive chromatography resins that have limited lifetime (although this is improving), high pressure requirements, and large amounts of labor and organic solvent usage. Any reduction in chromatography time translates into solvent, resin, and labor savings for the CMO. With **crude purities** for short peptides made using GAP-PS **averaging 90% or higher**, it is often practical to proceed directly to the "polishing step" after isolation of the crude peptide product. Even for products that do not require multiple chromatographic purification steps, a higher crude purity translates to improved efficiency across final purification. Additionally, for some peptide products with lower purity requirements, such as cosmetic peptides, chromatography may be bypassed entirely. This affords significant relief from the purification burden of peptide manufacture, allowing a reduction in solvent cost, and the opportunity to process more peptide products in a shorter period. This could potentially increase the capacity of a manufacturing facility and the high-level profitability of a CMO.

ii. Reduction of raw material consumption

Focusing on the hard costs of peptide manufacturing provides an opportunity to reduce raw material consumption, namely amino acids and coupling reagents. In a typical SPPS coupling reaction, an excess of amino acids and coupling reagents are used, sometimes up to 5+ equivalents for unoptimized processes, and up to fifty percent excess even for optimized syntheses;[5, 10] the excess can be necessary to drive the reaction to completion to avoid deletion sequences, which can then be problematic during purification and adversely affect yield. The need for large excess results, in part, from the nature of SPPS as a heterogenous reaction mixture. In contrast, emerging research results show GAP-PS needs no more than 2 equivalents of raw materials for any coupling attempted to date. Often, GAP-PS couplings can proceed to completion with as little as 1.1 equivalents or 10% excess **without optimization**. This benefit arises, in part, from the homogeneous solution-phase nature of GAP-PS reactions.

iii. Reduced solvent usage, waste, and cost

There are two steps in GAP-PS procedures that deliver an identified reduction of solvent costs. First, with GAP-PS' similarity to SolPPS processing, it uses less solvent than SPPS techniques. For example, the popular SPPS Sieber resin, with an average loading of 0.4 mmol peptide per gram of resin, would require 2.5 grams of resin to synthesize 1 mmol of peptide. The resin would be suspended and swollen in 25 mL DMF. For the coupling reaction, a solution of amino acids and coupling reagents (20 mL) must be added, followed by 3 X 20 mL washes to thoroughly remove impurities.[6-9] For the deprotection reaction, more solvent is required, using 2 X 20 mL deprotection solution in DMF, followed by 6 X 20 mL washes to completely remove the deprotection reagents. This brings the total solvent usage for the SPPS coupling/deprotection cycle to 265 mL. In contrast, GAP-PS is most often performed at approximately 50 mM concentration (although successful peptide synthesis has occurred at up to 100 mM concentration). For 1 mmol of peptide at 50 mM concentration, this equates to roughly 20 mL of solvent to dissolve the peptide, amino acid, and coupling reagents. After coupling, aqueous workup, deprotection, and another aqueous workup (all with roughly the same 20 mL of solvent), the peptide is precipitated using 40 mL of hexanes, totaling 60 mL of solvent for the GAP-PS coupling/deprotection cycle. This example demonstrates a reduction in solvent usage greater than 75%.

The second solvent cost reduction opportunity materializes because GAP-PS is run in solution, which gives greater flexibility in solvent choice, and therefore potential for cost savings. GAP-PS has been conducted successfully in dichloromethane along with greener solvents such as ethyl acetate, propylene carbonate, MTHF and others. This diversity is advantageous for manufactures needing method flexibility.

iv. Ease of chemistry

Peptide pharmaceuticals and use of peptides in cosmetics, nutritional supplements and a variety of other applications are on the rise. New routes of administration of these products are under increasing development[11] and often require higher doses of peptide;[12,13] as a result, peptide manufacturing technologies amenable to scaleup are becoming more and more desirable. Several attributes of GAP-PS appear to make the method an attractive option from a scaleup perspective:

- GAP-PS has proven to proceed with a variety of coupling and deprotection conditions and reagents, often at room temperature;
- the chemistry also functions in a variety of solvents, both heavier and lighter than water, allowing for adaptability in the process to fit the needs of specific peptide scaleup requirements;
- depending on the sequence and solubility characteristics, some peptides made with GAP-PS may be isolated as fine and workable powders without lyophilization;
- the adaptation of Fmoc/*t*Bu chemistry to the solution phase method via GAP-PS helps with reagent sourcing; due to the prevalence of Fmoc chemistry in peptide synthesis, convenient access to large quantities of necessary regents exists;[11]
- additionally, GAP-PS is not restricted to Fmoc chemistry and is amenable to other peptide synthesis schemes such as Boc, Cbz, and Trt.

Increasing Synthesis Efficacy:

Over the past year, GAP Peptides, LLC has been enhancing the technology and generating data to prove the fidelity and robustness of the process in addressing industry's concerns. With an initial focus on shorter peptide targets, GAP Peptides' Phase 1 substrate scope demonstrates the chemistry can handle a wide variety of different amino acids, different functionality, and different applications ranging from cosmetics to pharmaceutical. Broadly defined average efficiency and average purity across the target set of peptides is also an objective of this Phase 1 research.

Current data reflects GAP peptide synthesis as a complementary blend of solution phase and solid phase peptide chemistry, offering the efficiency and scalability of SolPPS while maintaining and improving on the purity and ease of SPPS. To assist in production of research data, GAPP has automated its novel process to run on a customized liquid handling system. This sophisticated system can synthesize up to 24 different peptide targets at once. Table 1 shown below is a subset of larger data that has been documented thus far.

Table 1: GAP-PS Substrate Scope

Peptide Name	Sequence	Crude Purity[1]
GHRP-6	HWAWFK	98%
Octreotide	FCFWKTCT[2]	88%
Substance P	RPKPQQFFGLM[2]	85%
EFK8	KFEFKFEF	90%
Pentapeptide - 1	KTTKS[3]	92%
GHK	GHK[3]	97%
Pal GHK	Pal-GHK[3]	95%
Oligopeptide - 20	CRKIPNGTYDTL[2,3]	95%
Pentapeptide - 3	GPRPA-(NH2)[3]	99%
Pentapeptide - 18	YAGFL[2,3]	83%
Pal tripeptide - 5	Pal-KVK[3]	99%
Pal dipeptide - 6	Pal-Lys-Val-Dab[3]	97%
Cyclotetrapeptide	LPAI[3]	98%
Thymopentin	RKDVY	99%
Pal tetrapeptide - 3	Pal-GQPR	86%

[1]No chromatographic purification; [2]Purity determined with GAP anchor still attached; [3]Synthesized on automated system designed for GAP-PS.

Quality, as reflected in high crude purities, is a desired outcome of innovative peptide production methods. GAP-PS research has resulted in peptides with an average crude purity of 92% without optimization. GAP Peptides can even achieve 99% crude purity on a few targets as can be seen in the example LCMS spectra in Figure 1.

Figure 1: LCMS Spectra of Crude Pal-KVK
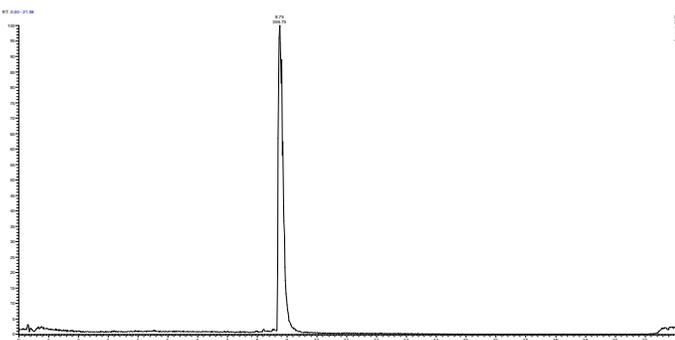
GAP Peptides, LLC continues to test and develop its process to respond to needs in the industry. Initial success has been recorded in the polycationic peptide arena; 8 to10-mer proprietary sequences - with more than 70% of the sequences carrying positive charges - have been synthesized at a crude purity of 83%, and research to increase this purity is ongoing. Longer sequences are also being attempted, with a goal of reaching upward of 30+ amino acids in length. Preliminary research has been conducted to synthesize multiple sequences on a multi-gram scale with promising results, and the company intends to scale the process with new projects in 2020. Critical to scaleup efficiency is crude peptide yield, which has been evaluated for a few targets with encouraging results. Table 2 is a small sample of recent yield results.

Table 2: GAP-PS Peptide Yield

Peptide Name	Sequence	Peptide Yield[1]
GHK	GHK[2]	71%
Pentapeptide - 1	KTTKS[2,4]	73%
Thymopentin	RKDVY[3]	86%
Pentapeptide - 3	GPRPA-(NH2)[2,4]	71%

[1]Yield based on amount of GAP anchor molecule used to begin synthesis; [2]Net crude peptide yield determined using % Nitrogen content of crude; [3]Gross peptide yield; [4]Data point updated or added to reflect recent results since initial publication on Dec 4th, 2019.

GAPP's future research and development focus forges into new grounds with its evolving technology, GAP-Lin*X*™. The base GAP-PS method uses a C-terminal protecting group that is orthogonal to the *t*Bu-based side-chain protecting groups commonly found in Fmoc amino acids. Instead of performing a separate reaction at the end of the synthesis to remove the GAP group from the peptide, GAP-Lin*X*™ technology eliminates this final step, allowing for a one-step global deprotection wherein the GAP molecule is removed along with the sidechain protecting groups. This approach additionally enables facile access to C-terminal amides: some GAP-Lin*X*™ protecting groups leave behind a C-terminal amide following global deprotection.

Conclusion

With a fully automated process and a growing data set of peptide examples, the current research reflects positively on the efficiency, repeatability, waste reduction, and optimization of process steps in the approach. Group Assisted Purification peptide synthesis holds promise that warrants consideration for sponsors and manufacturers seeking innovative options for more affordable peptide production methods. While research to expand the depth and breadth of the proof of concept continues, existing evidence suggests that GAP-PS could be a truly feasible approach offering manufacturers an alternative to 20th century methods.

FOOTNOTES & REFERENCES
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