GAPPeptides™

Transforming peptide synthesis for the 21st century

Group Assisted Purification Peptide Synthesis

GAP Peptides, LLC offers a cost-saving platform technology for large scale production of synthetic peptides. The patent-pending Group Assisted Purification Peptide Synthesis (GAPPS) method reduces raw material and solvent consumption by up to 50%; increases throughput, utilizes green chemistry, and minimizes waste. GAPPS allows lower-cost scale up while maintaining yield and high crude purity. To date, the technology has been applied to peptides used in the cosmetic and pharma industry and has been proven at a 100 g scale.

Sample of Small Scale / Short Targets (unoptimized):

Peptide Name	Sequence	Crude Purity	Application
GHRP-6	HWAWFK	98%	Hormone Treatment
Octreotide*	FCFWKTCT	88%	Anti-Cancer, Gastrointestinal
Substance P*	RPKPQQFF GLM	85%	Neurological Disorders
Oxytocin	CYIQNCPLG -(NH_2)	95%	Obstetrics
Pentapeptide -1	Pal-KTTKS	95%	Cosmetics
GHK	GHK	97%	Cosmetics
Pal GHK	Pal-GHK	95%	Cosmetics
Pentapeptide-3	GPRPA-(NH_2)	99%	Cosmetics
Pal Tripeptide-5	Pal-KVK	99%	Cosmetics
Pal Dipeptide-6	Pal-KV-Dab	97%	Cosmetics
EFK8	KFEFKFEF	90%	Cosmetics

** Purity determined with GAP anchor molecule still attached*

GAPPS uses solution-phase Fmoc/tBu synthesis methods in conjunction with Group-Assisted Purification (GAP) chemistry to achieve high crude-purity and yield.

The GAP anchor molecule replaces solid-phase resins. While GAP chemistry does not require precipitation or filtration, the GAP anchor can facilitate selective precipitation of the peptide at any time, allowing removal of organic byproducts and enabling a wide variety of possible chemistries.

Key Benefits of GAP Peptide Synthesis:

- Significantly **reduces material consumption** throughout the synthesis process
- **Reduced process cycle time** resulting in fast synthesis (≥ 3 couplings / day)
- High crude purities (up to 99% ***without*** column chromatography).
- **Easy, in-process controls** and analytics via direct reaction monitoring
- Significant **reduction of hazardous waste**
- **Green solvents**: 2-MeTHF, CPME – flexible for other solvent use

GAPPS: Cost Efficient, Green, Scalable

- ❑ Fast, easy synthesis of GAP-anchor molecule from inexpensive, commercially available raw materials
- ❑ GAPPS uses standard Fmoc chemistry, all steps are performed in solution
- ❑ GAPPS requires no special equipment or filters
- ❑ Minimal excess of reagents and solvents required for coupling completion
- ❑ Embraces principles of Green Chemistry: uses 2-MeTHF as solvent, CPME for precipitation following TFA deprotection
- ❑ GAPPS uses water washes (not solvent washes) to remove excess reagents
- ❑ Significantly reduces solvent consumption, waste, and disposal costs
- ❑ Homogenous, "one-pot" approach enables direct monitoring for in-process analytics
- ❑ Reactions are designed to be stable for up to 2 hours, providing a wide window for reaction monitoring and control
- ❑ Exceptional phase separation time shortens overall cycle time and, along with high reaction concentration, increases throughput
- ❑ GAPPS reaction temperature can be optimized (ambient or elevated) to balance throughput and energy costs

Sample Research Results: 10g & 100g synthesis using GAPPS:

Peptide	Crude Yield (Net, g)	Crude % Yield (Net per %N)	Crude Purity
Pal-KTTKS	13.54	93%	> 95%
Pal-KTTKS	121.40	90%	> 95%

Example: estimated materials consumed in SPPS vs. GAPPS for 5-mer cosmetic peptide:
Kg raw material & solvent/ Kg peptide product

Category1	SPPS (Kg)	GAPPS (Kg)	% Savings from GAPPS
Resin / Anchor	2.775	0.489	82%
Amino Acids	8.495	3.753	56%
Coupling Reagents	9.109	2.612	71%
Fmoc Deprotection Reagents	25.708	6.911	73%
Synthesis Solvent	798.073	134.704	83%
Final Deprotection: TFA and Solvent	108.004	89.249	17%
Chromatography Solvent	550.000	550.000	0%*
TOTAL	1502.163	787.717	48%

**Chromatography generally assumed to be the same; however, GAPPS often delivers higher crude purity than SPPS, which can result in significant reduction of chromatographic steps and solvent - depending on the target and impurity profile.*

GAPPS a Cost-Efficient Platform Technology

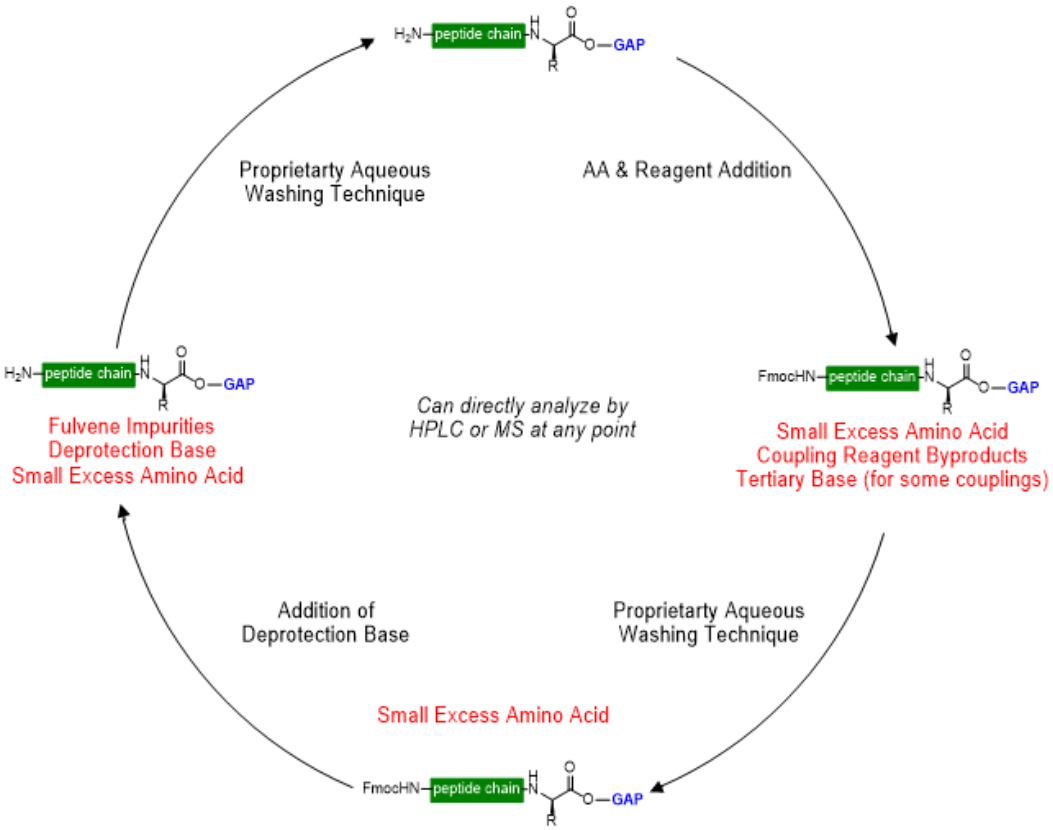
Savings	Time	Money	Energy	Reduce Waste	Reduced Footprint
Eliminate bulky, expensive SPPS resins		✓		✓	✓
NO specialized equipment required		✓			✓
Minimize raw material consumption		✓		✓	
High crude purity reduces purification process	✓	✓	✓	✓	
Aqueous extraction **eliminates the need for in-process filtration**	✓	✓	✓	✓	✓
Minimize solvent use and solvent waste; Use of **green solvents** minimize hazards and reduce environmental impact		✓	✓	✓	
Standard in-process controls using standard analytical chemistry - without degradation or waste of product	✓	✓	✓		
High reaction concentration **increases process throughput**	✓	✓	✓		✓
Reaction temperatures easily optimized to balance throughput and energy costs		✓	✓		
High **process yield –**more product, less resource consumption		✓	✓	✓	✓

GAPPS combines the advantages of SPPS & LPPS and enhances them:

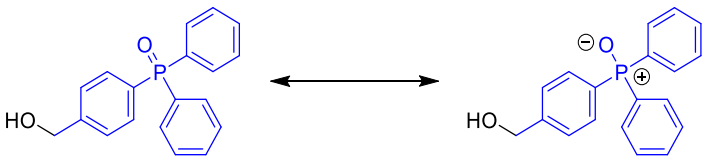
Comparing LPPS v SPPS v GAPPS	LPPS	SPPS	GAPPS
Reaction medium	Solution	Gel (swollen resin)	**Solution**
Synthesis strategy	Convergent or Step	Step	**Either**
Temporary protecting group	Boc or CbZ	Fmoc	**Either**
Speed of coupling completion	Slow	Fast	**Fast**
Consumption of raw materials	Moderate	Medium to high	**Minimal**
Consumption of organic solvent	Moderate to medium	High	**Minimal**
In-process control	Direct monitoring	Indirect monitoring	**Direct**
Isolation of intermediates	Precipitation	Not required	**Not required but possible anytime**

GAP Peptides' international IP portfolio offers a flexible pathway for commercialization via sub-licensing. Contact the team at GAP Peptides to learn more: info@gappeptides.com

GAP Peptide Synthesis General Methodology



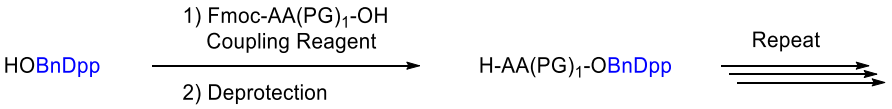
The GAP Anchor: Composition and Application

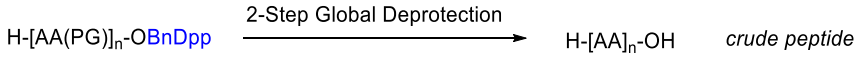


HOBnDpp
1st Generation GAP Anchor

Poor P-orbital overlap, leads to:
Strong ionic character, leads to:
Solubility control for solution phase

Small Molecule, leads to:
High peptide loading, greater throughput
Inexpensive Anchor Synthesis





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