

**BACHEM  
PEPTIDE  
GUIDE  
BACHEM**

**LEADING PARTNER IN TIDES**

# A BRIEF INTRODUCTION TO SYNTHESIS, DESIGN, AND HANDLING OF PEPTIDES

## Table of Contents

Peptide Synthesis	3
The Principle of Solid-Phase Peptide Synthesis (SPPS)	3
Peptide Purification	5
Quality Control of Peptides	5
How to Design Your Custom Peptide	8
Amino Acids Prone to Undergo Side Reactions	9
Peptide Modifications	11
Care and Handling of Peptides	14
Delivery Time	15
Most Frequently Asked Questions	16
Questions Related to the Calculation of Prices	17
Questions Related to Quotation Inquiries and Orders	17
Questions Related to the Synthesis	18
Questions Related to Purity and Analytical Methods	19
Questions Related to Handling and Storage	20
Conclusion	21
Abbreviations	22

With over four decades of experience in peptide synthesis and the world's largest group of peptide chemists in the industry, Bachem is your ideal partner for custom synthesis, catalog peptides, and complex organic molecules. We offer full range of technologies which are available at our production sites in the USA and in Europe. We produce research grade peptides as well as GMP-grade material, from simple peptides to the most complex peptidomimetics or synthetic proteins. Our experts will support you in the design of your peptides and peptide derivatives. The aim of this guide is to present a general survey of the methods of peptide production, and to provide answers to the most frequently asked questions by the end user. This publication is focused on peptides used for research purposes (i.e. milligram to gram-scale).

## Peptide Synthesis

Peptides can be obtained chemically by solution-phase synthesis, by solid-phase peptide synthesis (SPPS), or by a combination of both methods, which can involve various ligation strategies. Normally, at Bachem, the synthesis of peptides is carried out on solid phase, whereas the classical approach is chosen for synthesizing di- and tripeptides, and, occasionally, C-terminally modified peptides such as enzyme substrates.

In the following paragraphs we will discuss the solid-phase approach in more detail, as this methodology is of utmost importance for the synthesis of peptides.

### The Principle of Solid-Phase Peptide Synthesis (SPPS)

During solid-phase peptide synthesis, a peptide which is anchored by its C-terminus to an insoluble polymer is assembled by the successive addition of protected amino acids constituting its primary structure. Hence, the peptide is elongated in the C to N direction (see General Scheme of Fmoc-SPPS on p. 5).

A synthetic cycle consists of:

- Cleavage of the  $\alpha$ -amino protecting group

- Washing steps to remove the cleavage reagent
- Coupling of the protected amino acid
- Washing steps to remove excessive material

As the growing chain is linked to an insoluble support excesses of reagents and by-products can be removed by repetitive washings with appropriate solvents. Only solvents which swell the peptide resin properly can be used for deprotection and coupling, whereas the washing protocol may include shrinking steps.

After completion of the synthesis, the desired peptide is cleaved from the resin. Usually, this cleavage step is performed with acids of varying strength.

Peptides are synthesized from the C-terminus to the N-terminus of the sequence.

### The Solid Support

Polystyrene, crosslinked with 1% divinylbenzene, is still the most popular carrier resin in SPPS. It is chemically inert under the conditions of SPPS, and it is readily derivatized allowing the introduction of a large variety of anchoring groups. The resulting resin swells sufficiently in solvents suitable for SPPS. The choice of the anchoring moiety is determined by the chosen synthetic strategy and by the type of C-terminus of the desired peptide.

As the necessary know-how and the required equipment for performing Fmoc and Boc syntheses are available at Bachem, the synthetic strategy for your peptide can be optimized.

Bachem has already succeeded in the synthesis of very complex peptides, which could not be produced elsewhere.

### Protecting Groups

Two categories of protecting groups are required for synthesizing peptides: groups allowing temporary protection of the  $\alpha$ -amino group and “permanent” protecting groups blocking the side-chain functionalities of the amino acids. The latter groups have to withstand conditions of repetitive  $N^{\alpha}$ -deprotection; usually, they are removed only during cleavage from solid support. Untimely removal of protecting groups is a common cause for the formation of by-products.

The best strategy to avoid this risk consists of introducing temporary and permanent protecting groups, which can be removed by differing chemical mechanisms, i.e. orthogonal protection.

Truly orthogonal protecting groups may be split off with absolute selectivity and in any order. The “classical” Boc/Bzl-strategy does not fulfill this requirement, as both groups are cleaved with acid.

However, their acid lability differs sufficiently to afford selective removal of the  $\alpha$ -amino protection. The combination Boc/Bzl may be called quasi-orthogonal. The pairing Fmoc/tBu, on the other hand, is truly orthogonal. The temporary  $\alpha$ -amino group is deblocked with base (piperidine). Thus, TFA-labile and simultaneously base-stable groups as tBu and Boc (in combination with a TFA-labile anchor) are the perfect choice for side-chain protec-

tion. Orthogonal protection schemes permit milder overall reaction conditions as well as the synthesis of partly protected or side-chain modified peptides.

### Fmoc/tBu or Boc/Bzl Strategy

The Boc/Bzl-strategy can be traced back to the beginnings of SPPS, Merrifield’s pioneering work. This methodology requires anchoring groups, which tolerate repetitive TFA treatment. Usually, the inorganic acid HF is employed for the final cleavage, which limits the batch size in this step and the choice of reactor. Even though many remarkable synthetic successes employing Boc/Bzl-technology are recorded in the literature, the development of orthogonal protection schemes increased the flexibility of the solid-phase method. The Fmoc/tBu-strategy is the most popular amongst them. It can be automated far more conveniently than the Boc/Bzl-strategy and it can be scaled as needed. Additional levels of orthogonality allow the synthesis of highly complex peptides. Nevertheless, depending on the sequence, the Boc/Bzl-strategy still can remain a viable alternative.

Fmoc	Boc
Routine synthesis	Requires special equipment
Acid-sensitive peptides and derivatives, e.g. O-glycosylated or sulfated peptides	Base-labile peptides; „difficult sequences“ (aggregation impeded by repetitive TFA-treatment)

a)  
Protected resin  
(color test negative)



b)  
Resin after removal of the protecting groups  
(color test positive)



### Long Peptides (up to 100 Amino Acids)

The demand for long peptides (up to 80-100 amino acids) is increasing. Such large molecules could be successfully synthesized at Bachem by stepwise SPPS following the described strategies. However, with increasing peptide length, this standard approach may fail. One possible synthetic methodology would be the use of Native Chemical Ligation (NCL) developed by Kent et al. as a viable alternative to stepwise SPPS for synthesizing very long peptides. Synthetic strategies comprising stepwise elongation of the peptide may yield a very impure crude product, which cannot be purified by

standard chromatographic protocols. The chemoselective coupling of unprotected peptide fragments is the essential feature of NCL, thus subsequent purification is reduced to removing unreacted fragments. The required segments are obtained by SPPS. Even the chemical synthesis of small proteins has become feasible, at least research quantities (10-20 mg) could be obtained employing a combination of stepwise SPPS and chemical ligation. The synthesis of proteins by this convergent approach is a viable alternative to standard recombinant technologies offering a plethora of additional options.

### Peptide Purification

The properties of an individual peptide depend on the composition and sequence of amino acids.

Acidolytic cleavage following SPPS yields a crude product containing the desired peptide and impurities such as deletion peptides, truncated peptides, incompletely deprotected peptides, modified peptides, scavengers and by-products derived from the cleaved protecting groups. All these contaminants have to be removed. Purification of synthetic peptides is routinely carried out by reversed-phase high performance liquid chromatography (RP-HPLC) using  $C_{18}$ -modified silica as the stationary phase and UV peak detection.

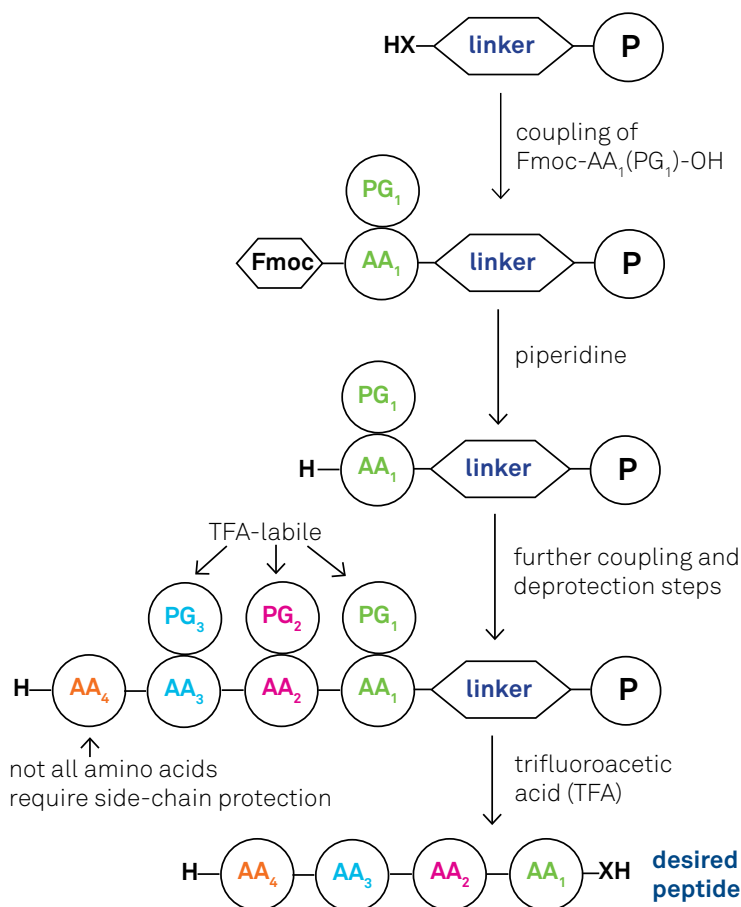
Target peptide and impurities are retained by the stationary phase depending on their hydrophobicity. Very polar contaminants will elute at the beginning with aqueous 0.1% TFA, then the polarity of the eluent is gradually reduced by continuously increasing the proportion of the less polar modifier, acetonitrile (a linear gradient is formed, the concentration of TFA is kept constant). The elution of material is monitored at 210 - 220 nm. Fractions containing sufficiently pure target peptide, as determined by analytical HPLC, are pooled and lyophilized.

If the desired compound cannot be obtained sufficiently pure by RP-HPLC applying the standard TFA-system, an appropriate combination of buffer systems will be developed. If the  $C_{18}$  stationary phase is too hydrophobic, e.g. when purifying less polar peptides, other column packing materials are selected.

### Quality Control of Peptides

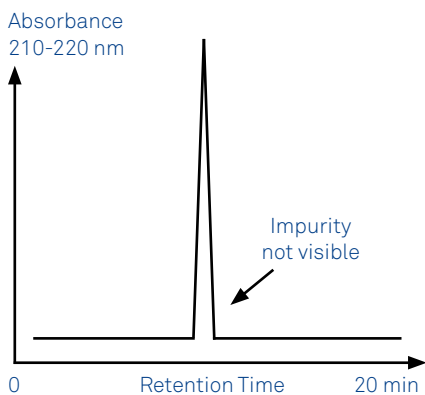
#### Definition of Peptide Purity

The purity of the lyophilized target peptide is determined by analytical RP-HPLC followed by UV detection at 210 - 220 nm. It is quantified as area percentage, as it corresponds to the area of the main peak in relation to the total area of all peaks, i.e. all material (including the requested peptide) which absorbs at this wavelength.

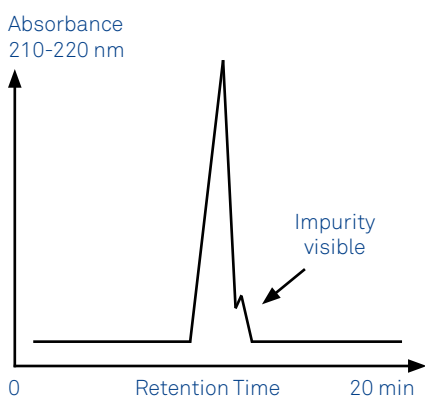


General scheme of Fmoc-SPPS  
X = O, NH  
AA = Amino Acid  
PG = Protecting Group  
P = Polymer Support

If two purity values are obtained using different analytical systems, we report the final purity as the lower of the two.



a) Steep gradient



b) Shallow gradient

Amide bonds and other chromophores absorb at approximately 220 nm, whereas water and residual salts are not detected UV-spectrophotometrically. The ability of this method to detect and quantify impurities eluting in the proximity of the product peak, i.e. an adequate resolution, is essential. The resolution of analytical HPLC can be improved by judicious choice of the buffer system, the stationary phase, the steepness of the gradient, the column temperature and other parameters. A small change of one of these parameters may turn a barely resolved shoulder into a closely eluting peak which can be integrated and thus quantified. This is demonstrated by the HPLC-profiles above. Bachem has over 40 years experience in the analysis of synthetic peptides using cutting edge HPLC equipment. Moreover, all analytical HPLC instruments used in production and QC are subjected to daily system suitability testing to ensure the veracity of analytical data obtained.

At Bachem, peptides used for quantitative studies are always provided with their Net Peptide Content.

### Definition of the Net Peptide Content (NPC)

Lyophilizates of peptides contain varying amounts of non-covalently bound water. Normally, the peptide is delivered as the TFA salt which results from the RP-HPLC purification. The side-chain functionalities of Arg, Lys and His and the free N-terminus will form trifluoroacetates and small amounts of TFA may adhere to the peptide. These contaminants cannot be detected by analytical HPLC.

Other salt forms of your peptide (e.g. acetate, hydrochloride) will be produced upon special request.

If not requested otherwise, peptides are isolated and provided as trifluoroacetates containing residual water. In accordance with the number of basic functionalities present in the peptide, they may contain a considerable number of counter-ions. Besides, lyophilizates of such salts are rather hygroscopic. Both water and counter-ions reduce the net peptide content.

The net peptide content is defined as the percentage of peptides relative to non-peptidic material, mostly counter-ions and moisture.

Net peptide content and purity are not equivalent, as the NPC includes peptidic contaminants. A low NPC has to be expected for peptides containing a large proportion of basic amino acids, even if they are extremely pure. Hydrophilic peptides can absorb considerable amounts of moisture.

$$\text{Absolute quantity of peptide} = \text{Quantity of lyophilizate} \times \frac{\text{Purity(\%)} \times \text{NPC(\%)}}{10\,000}$$

The NPC can vary from batch to batch, depending on the conditions of final purification and lyophilization.

The NPC is determined by amino acid analysis or elemental analysis (CHN), as the non-peptidic contaminants do not contain nitrogen. Net peptide content and purity have to be taken into consideration when preparing solutions of biologically active peptides for assays.

### Impurities

After isolation and purification impurities may still contaminate the peptide, amongst them deletion sequences (peptides lacking at least one of the required amino acids), incompletely deprotected sequences, truncated peptides, by-products formed during peptide synthesis or under the conditions of cleavage.

Except for TFA, all potentially cytotoxic reagents used in the course of the synthesis should have been removed by the washings preceding the final cleavage or during the purification process. Traces of residual solvents can be determined by gas chromatography (GC) if required.

### Batch-to-batch Variability of Peptides

The purity of a peptide, i.e. the proportion of desired product, can vary from batch to batch. When a peptide is ordered at 80% purity, the quality of the product can range between 80% and 100%. The lower the requested purity, the broader the observed variability between two lots. Hence, results obtained from quantitative assays could vary unpredictably depending on the quality of the particular batch. Batches of low purity contain a considerable number of peptidic by-products. Proportion and structure of these contaminants will vary from batch to batch. Peptidic impurities may show biological activity as well, but not necessarily the activity of the target peptide. In the worst case they may interfere with the assay.

The NPC can vary as well. It is influenced by the polarity of the peptide, the conditions of lyophilization, the conditions and duration of storage, the contact with humidity and many other parameters. Unpurified peptides should not be used in biological assays, even if the assay could be conducted employing a low-purity product. The material obtained after cleavage from the resin and precipitation still may contain a range of harmful non-peptidic impurities, e.g. small amounts of scavengers. Fortunately, peptides which were purified by standard procedures and lyophilized will contain only traces (in the ppm-range) of cytotoxic non-peptidic contaminants (such as residual solvents and scavengers from cleavage). Only TFA cannot be removed

completely due to salt formation. If residual TFA may pose a problem, we recommend ordering a more biocompatible salt form of the active peptide.

However, as an additional ion exchange step will be required, the price of the custom peptide will have to be adjusted.

All of our custom peptides from Immunograde to > 95% are purified by RP-HPLC.

### Recommended Peptide Purity for Varying Applications

Four standard product grades are offered by Bachem and intermediate purity ranges can be provided on demand. The lower the level of purity, the lower the price will be. The correlation between purity and price is not linear. Efforts and costs for obtaining very pure peptides (97-99%) may increase exponentially.

Purity	Applications
> 95%	<ul style="list-style-type: none"> <li>• NMR studies</li> <li>• Crystallography studies</li> <li>• Peptides used as reference in final quantitative studies:               <ul style="list-style-type: none"> <li>* Enzyme-substrate studies</li> <li>* Receptor-ligand interaction studies</li> <li>* Blocking and competition assays</li> </ul> </li> </ul>
90 - 95%	<ul style="list-style-type: none"> <li>• Production of monoclonal antibodies</li> <li>• Enzyme-substrate studies (quantitative)</li> <li>• Receptor-ligand interaction studies (quantitative)</li> <li>• Blocking and competition assays (quantitative)</li> <li>• ELISA and RIA (quantitative)</li> <li>• In vivo/in vitro studies</li> </ul>
> 80%	<ul style="list-style-type: none"> <li>• Western blotting studies (non-quantitative)</li> <li>• Enzyme-substrate studies (non-quantitative)</li> <li>• Phosphorylation studies</li> </ul>
Immuno-grade (> 65%)	<ul style="list-style-type: none"> <li>• Production of polyclonal antibodies</li> <li>• Determination of the titer of antibodies in standard ELISA</li> </ul>

All custom peptides at Bachem are manufactured to the same high standards of quality for which Bachem set the benchmark over the past 40 years. Our technical experts will be pleased to answer your questions concerning your Analytical Data Sheet (ADS).

### Peptide Analysis

Bachem's custom peptides are, depending on the requested purity grade, accompanied by the analytical data obtained by HPLC and MS. Determination of the net peptide content (NPC) is supplied on request.

**HPLC:** The purity of the peptide is determined by RP-HPLC. The chromatogram additionally indicates the number and relative amount of by-products.

**MS:** The molecular mass of the peptide is determined by mass spectrometry to confirm that the correct product will be delivered. Moreover, the mass spectrum displays the masses of the main impurities.

Bachem routinely performs ESI-MS (electrospray ionization) and MALDI-TOF-MS (matrix-assisted laser desorption ionization-time of flight).

**NPC:** The net peptide content is assessed by amino acid analysis (AAA) and/or by elemental analysis, as it corresponds to the nitrogen content of the peptide. Additionally, AAA allows to verify the amino acid composition of the peptide. Especially for short peptides, e.g. enzyme substrates, elemental analysis replaces AAA as an additional confirmation of identity.

### How to Design Your Custom Peptide

When conceiving a peptide sequence for custom synthesis, the feasibility of its synthesis has to be kept in mind. A range of factors influences the outcome of a peptide synthesis and the properties of the target peptide including its stability. These aspects should be considered before definitively placing an order for a custom synthesis.

Our experts will support your search for an optimal but feasible sequence without additional charge.

### Length of Peptide

As the number of potential by-products grows with each additional step, the purity of the crude peptide decreases with increasing length. Nevertheless, many exceptions to this rule can be found in the literature.

Although quite a few examples for the synthesis of peptides containing up to 100 residues have been published, the solid-phase synthesis of very long peptides still presents a challenging task.

At Bachem, each sequence is evaluated by our chemists before quotation. They will inform you about potential problems associated with your peptide design. If unexpected difficulties occur during synthesis or purification we will inform you, especially if the agreed purity cannot be attained.

The synthesis of short peptides consisting of less than 5 predominantly hydrophobic amino acids may pose a problem as well, as such molecules are hardly soluble. Hence, purification is impeded.

The subdivision into small, medium-sized and long peptides (eventually, small proteins) is quite arbitrary. An approximate classification is summarized in the scheme on p. 9.

### Polarity

The solubility of a peptide in aqueous systems and, consequently, the ease of purification by reversed phase HPLC are strongly dependent on the overall amino acid composition.

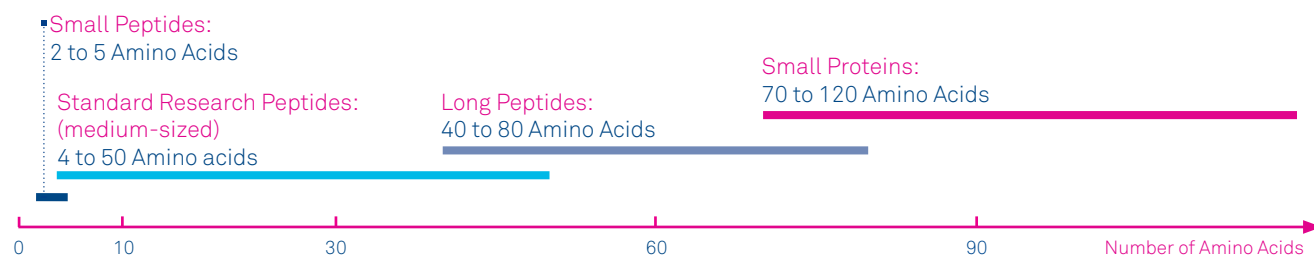
The proteinogenic amino acids can be divided into the four groups shown below: basic, non-polar/hydrophobic, polar/un-

We must emphasize that standard custom peptides are not suitable for human use.

Peptides intended for use in humans have to be synthesized in a cGMP environment.

Production under cGMP conditions has to be requested explicitly. Bachem is the world leader in the production of cGMP peptides including the required documentation.





charged and acidic (see also the *Periodic Chart of Amino Acids* at p.23 for a visual representation).

Peptides containing a large proportion of basic and acidic amino acids are readily soluble in aqueous buffers at physiological pH (pH ~ 7), whereas a large number of basic residues facilitates the dissolution in acidic solvent systems such as 0.1% aqueous TFA used for preparative chromatography. A large proportion of polar amino acids will improve the solubility of the peptide as well. The insertion of a Pro in the sequence may break a secondary structure or disrupt aggregation. Both effects increase the solubility.

The presence of Pro residues also facilitates the SPPS of sequences which would aggregate otherwise.

#### Classification of Amino Acids

Basic	Arg, His, Lys
Non-polar, hydrophobic	Ala, Ile, Leu, Met, Phe, Pro, Trp, Val
Polar, uncharged	Asn, Cys, Gly, Gln, Ser, Thr, Tyr
Acidic	Asp, Glu

A close look at the sequence will allow a rough prediction of polarity and solubility of the peptide and thus, the anticipation of problems during synthesis and purification. Difficulties can be expected when synthesizing peptides containing a large proportion of non-polar amino acids. Practically insoluble products may result, which can be purified only with great difficulties. The conservation of the biological activity limits solubilizing modifications to a peptide. But merely a minor reduction of length

or the incorporation of charged residues at the termini may help to avoid the predicted difficulties. This aspect has to be kept in mind when selecting partial sequences of a protein for custom synthesis

Bachem scientists are the leading experts for producing research catalog and custom peptides and small proteins.

#### Amino Acids Prone to Undergo Side Reactions

##### Amino Acids Sensitive to Oxidation

**Met, Trp** and, in particular, free **Cys** residues are susceptible to oxidation. Hence, peptides containing these amino acids have to be handled with appropriate care. They should be dissolved only in carefully degassed solvents.

The oxidation of a Cys-containing peptide yields a disulfide bridge i.e. a cystine peptide, Met is converted into a sulfoxide. Both transformations are reversible. Inter- and intramolecular disulfide bonds between the Cys thiol groups are formed very rapidly at pH >7. The bridging may be reversed by treatment with reducing agents such as dithiothreitol (DTT). Hence, peptides containing free cysteine residues should be dissolved in buffer systems including a reductant. If a cysteine is not absolutely required for the biological activity, it can be replaced by the hydrophobic isostere Abu ( $\alpha$ -aminobutyric acid) or by the polar Ser. The latter may participate in reactions of the native peptide.

Met can be replaced by the inert isosteric norleucine (Nle) residue. Both amino acids are hydrophobic. In most cases, the biological activity of the peptide remains unchanged. On the other hand, the polarity of the peptide is slightly increased by the oxidation of the thioether. The biological activities of reduced and oxidized peptide often vary; interesting effects may be gener-

ated by this readily available modification. **Tyr**- and especially **Trp**-containing peptides should be protected from intense sunlight, as both amino acids are susceptible to photo-oxidation. Oxidation of the lateral phenol and indole moieties by oxygen radicals is a rather common post-translational modification of proteins. Additionally, the indole ring is acid-sensitive.

**Asp**-containing peptides are susceptible to acid-catalyzed aspartimide formation. The ease of cyclization markedly depends on the nature of the subsequent amino acid. In case of Asp-Pro the peptide is cleaved. The motifs Asp-Gly and, to a lesser degree, Asp-Ser are especially prone to aspartimide formation. The subsequent hydrolysis of the ring yields a mixture of the  $\beta$ -Asp peptide and the native sequence. The concomitant racemization of Asp aggravates the situation. Aspartimide formation is equally involved in the base-catalyzed deamidation of **Asn**.

N-terminal **Gln** shows an extreme tendency to form cyclic pyroglutamate (Pyr). N-terminal acylation will suppress this side reaction. When coupling a Pyr derivative instead of Gln, a better defined product is obtained. The peptide is stabilized by an N-terminal Pyr, a very common feature in bioactive peptides.

Substance P is a perfect model for N-terminal degradation by diketopiperazine formation during storage. This side reaction may occur, if a Pro follows the N-terminal amino acid and especially, if the amino acids adjacent to this residue are unhindered (e.g. Gly).

### $\beta$ -Sheet Formation

Even though  $\beta$ -sheet formation cannot be categorized as a side reaction, it has to be mentioned in this context, as it is the cause of many problems occurring during synthesis and handling of a peptide. Incomplete solvation of the growing peptide chains due to  $\beta$ -sheet formation during SPPS leads to formation of deletion sequences and other by-products.

As demonstrated by structural analysis of model peptides forming  $\beta$ -hairpin structures in aqueous solution, a range of amino acids shows a propensity to be incorporated in  $\beta$ -sheets. Gln, Ile, Leu, Phe, Trp, Thr, Tyr,

and Val rank highest among them. Hence, peptides containing a large proportion or clusterings of these amino acids may show the tendency to aggregate. Naturally, the sequence will influence the extent of  $\beta$ -sheet formation as well, and the solubility of the peptide. The "conservative" replacement of Gln by Asn can help, Thr may be substituted by Ser. A slightly altered choice of the partial sequence of a protein to be synthesized may result in reduced aggregation. As Pro residues are the most efficient means to disrupt secondary structures, pseudoproline derivatives have been introduced for facilitating SPPS. These derivatives are obtained from Ser or Thr, which limits their use to the synthesis of peptides containing a Ser or Thr in suitable positions. They are introduced as dipeptides, Fmoc-Xaa-psiPro-OH, which will increase the production costs substantially. Ser or Thr are regenerated during acidolytic cleavage. The purity of the raw material will be considerably higher which simplifies the subsequent purification. Methods for obtaining solubilized peptide derivatives withstanding the cleavage from the resin are under investigation.

Bachem is one of the few suppliers of Fmoc-Ser(GalNAc(Ac)<sub>3</sub>- $\alpha$ -D)-OH, Fmoc-Thr(GalNAc(Ac)<sub>3</sub>- $\alpha$ -D)-OH, and the corresponding  $\beta$ -GalNAc and  $\beta$ -GlcNAc derivatives which can be employed for obtaining glycopeptides.

## Peptide Modifications

A choice of modifications of peptides available is listed below:

- Labeling with stable isotopes (<sup>15</sup>N, <sup>2</sup>H, <sup>13</sup>C)
- PEGylation
- Acetylation, acylation (e.g. lipopeptides)
- Biotinylation
- Amidation
- Backbone modifications as
  - hydroxyethylene isosteres
  - thioethers
  - retro-inverso peptides
  - reduced peptide bonds
- Conjugation to carrier proteins (KLH, BSA, OVA)
- Conjugation to small molecules (including imaging agents)
- Conjugation to polymers
- Cyclizations
  - single or multiple disulfide bridges
  - head-to-tail
  - side chain (e.g. lactam bridge, thioether)
- Depsipeptides
- Hydrocarbon-stapled peptides
- Introduction/incorporation of
  - chelating moieties
  - chromophores and fluorophores
  - fluorophore/quencher pairs
  - D-enantiomers
  - maleimido groups
  - α- and N-methylamino acids
  - unusual amino acids
- Peptide alcohols and aldehydes
- C-terminal esters and thioesters
- Phosphorylation and sulfation

Acetylation, amidation and biotinylation are most frequently requested by our customers.

As these modifications only minimally increase our input, they can be offered as routine operations, which will not be charged additionally.

If you are interested in a modification not mentioned in this compilation, please inquire.

### Conjugation to Carrier Proteins

Three standard proteins are used at Bachem as carriers for peptides: KLH, BSA and OVA. Usually, the peptide is coupled to

the protein either via its N-terminus or via the SH functionality of a cysteine. Conjugation via a thiol group is the preferred method as it is highly selective. Thus, an additional Cys is coupled either to the C-terminus, or, more practically, after completion of the SPPS if the desired peptide lacks this amino acid. The addition of an N-terminal Cys allows obtaining the Cys-derivative required for the conjugation together with the peptide lacking the Cys (for binding assays) from the same batch. Finally, as the yield of peptide conjugation to the carrier protein is critically important for the analysis of variations in immune response, Bachem may, upon request, incorporate an unnatural amino acid into the peptide hapten for quantitation of conjugation efficiency.

### Unusual Amino Acids

α-Amino acid bearing unusual side-chain functionalities and turn mimetics are useful tools for peptide design.

Bachem offers an exceptional choice of unusual amino acids. Most of them are available as N<sup>α</sup>-protected derivatives from stock, so they can be inserted into your peptide without delay.

Amino Acids	Examples
Modified Standard Amino Acids	D-Amino acids N-Methylamino acids
Unusual Amino Acids	Citrulline δ-Hydroxylysine β-Alanine
Unnatural Amino Acids and Building Blocks	4-Fluoro-phenyl-alanine Norleucine β-Mercaptopropionic acid

### Disulfide Bridges

The synthesis of peptides containing three or more disulfide bridges requires special care to ensure the homogeneity and predicted bioactivity of the purified synthetic product. Two strategies are commonly employed for this purpose: oxidative folding and 'directed' disulfide formation. Oxidative folding is essentially a 'biomimetic' process by which the reduced precursor sequence is folded under near-physiological conditions to yield the thermodynamically stable oxidized product. Directed disulfide formation, on the other hand, uses unambiguous

Bachem offers the largest range of special amino acids available for custom peptide synthesis. Please contact us for a poster Periodic Chart of Unusual α-Amino Acids.

Bachem has an excellent reputation for synthesizing peptides containing multiple disulfide bridges.

Bachem has gained extensive know-how in synthesizing peptides containing two to three phosphorylated residues.

chemical methods in a stepwise manner to prepare the product with the desired disulfide connectivity, which is 'programmed' into the synthetic route. Both methods have advantages and disadvantages. They have been implemented at Bachem on mg to kg scales for the synthesis of a wide range of targets.

When required, these chemistries are always discussed in technical detail to identify the most appropriate strategy for a given custom synthesis project.

#### **Cyclization via Amide Bond**

Besides disulfide bridging, cyclization of a peptide can be achieved by other methods. "Head-to-tail cyclization", an amide bond formation between the N- and the C-terminus of the peptide, can be achieved following SPPS of the side-chain protected peptide. Stabilization of a desired conformation is gained by the side-chain cyclization of  $\omega$ -amino and  $\omega$ -carboxy groups, which requires an additional level of orthogonality. Such lactam bridges are formed between Asp/Glu/Aad/Asu and Dap/Dab/Orn/Lys, i.e. ring size, flexibility and the direction of bond formation can be varied. Additionally, these amide bonds are more stable than disulfide bridges. If required, other modes of cyclization (e.g. formation of thioethers, metathesis) can be performed.

#### **Phosphopeptides and Sulfopeptides**

O-Phosphorylation and O-sulfation are very common post-translational modifications of proteins, and are commonly requested for synthetic peptides. However, the chemical stability of phosphorylated and sulfated peptides requires the use of specialized tactics during synthesis and purification of these molecules. At Bachem, several synthetic methodologies are commonly used for these targets, usually based on pre-derivatized building blocks to minimize the incidence of side reactions and ensure site-specific incorporation. Nonetheless, these projects require careful attention during manufacturing and handling upon receipt by our customers, and we are always happy to provide guidance on stability optimization and recommendations

Bachem offers a vast choice of chromophores and fluorophores for the design of enzyme substrates, dye-labeled peptides, and FRET technology. Our experience and chemical know-how allows us to synthesize all types of inhibitors.

to ensure the integrity of the target molecule during testing.

#### **Glycopeptides**

N- and O-Glycosylations of proteins are important post-translational modifications as well. Glycosylated peptides may act as a stimulator in the immune system. The demand for such compounds is constantly increasing even though their synthesis still represents a very challenging task. The synthetic problems are due to a variety of reasons such as the limited choice of protecting groups for the glycoside moiety and the high lability of the O-glycosidic bond.

#### **Lipopeptides**

Substantial difficulties have to be expected during the purification of lipopeptides due to their increased hydrophobicity. Before starting the synthesis of the lipopeptide, the sequence to be lipidated is studied carefully to determine the most suitable position for the introduction of the lipid moiety. A considerable number of palmitoylated peptides has been successfully synthesized at Bachem. The lipophilic cysteine derivatives Fmoc-Pam<sub>2</sub>Cys-OH and Pam<sub>3</sub>Cys-OH are available from stock. These compounds, which resemble the N-terminus of the lipoprotein from the outer membranes of *E.coli*, have been used at Bachem for synthesizing immunogenic conjugates such as peptide mitogens or vaccines.

#### **Peptides Labeled with Stable Isotopes**

The production of peptides labeled with stable isotopes such as <sup>13</sup>C, <sup>15</sup>N or <sup>2</sup>H is limited only by the commercial availability of the correspondingly labeled amino acids. Isotope labels are especially useful in NMR studies of peptides. A range of protected <sup>15</sup>N-labeled amino acids is available from stock at Bachem. Further labeled amino acids will be acquired, if they are commercially available, for the synthesis of suitably protected derivatives. The price of the peptide will have to be increased in accordance with our expenses.

### Fluorophores and Chromophores

A broad range of derivatives of fluorophores and chromophores suitable for the labeling of peptides is available to meet our customers' requirements. In most cases, the dyes are introduced either N-terminally or C-terminally. The synthesis of a C-terminally labeled peptide usually is more complex than obtaining an N-terminal modification. The incorporation of a label at the N-terminus merely means an additional step in the SPPS protocol, even though more elaborate coupling procedures may be required. The expense of many fluorescent dyes requires that specialized coupling protocols be used in order to maximize reaction yields with fewer stoichiometric equivalents of these costly raw materials. Additionally, dyes can be linked to the peptide by selective reaction with a cysteine thiol moiety or the less hindered  $\epsilon$ -amino group of lysine. The insertion of a spacer moiety between the dye and the peptide helps to avoid interactions between the label and the peptide, which will help retain conformation and biological activity. Additional effects may be attained by varying the length, the flexibility and the hydrophilicity of the spacer. The flexible non-polar  $\omega$ -amino carboxylic acids, e.g.  $\epsilon$ -aminocaproic acid, and the hydrophilic 2-[(2-amino)-ethoxy]-ethoxy-acetic acid (AEEAc) are readily coupled to the N-terminus or to the Lys side-chain.

When devising FRET substrates, the Förster distance, i.e. the distance between fluorophore and quencher allowing an efficiency of energy transfer of 50% (usually 20-90 Å), has to be achieved at the minimum to obtain a good quenching effect. It depends on the type of fluorophore/quencher pair. Only a limited number of amino acids can be inserted between dye and quencher moiety, otherwise the background fluorescence may reach unacceptable levels. The incorporation of a flexible spacer may disturb the energy transfer. For more detailed information, please visit our website or ask for our Technical Note *Chromophores/Fluorophores: Spectral Properties and Characteristics* and our brochure *FRET Substrates*.

### Enzyme Substrates and Inhibitors

C-terminal chromophores and fluorophores such as pNA and AMC are applied in substrates for the detection and quantitation of enzymatic activity. A different type of C-terminal residue is required to turn a substrate interacting with the active center of the enzyme into an inhibitor binding reversibly or even irreversibly to this site. The introduction of aldehyde, hydroxamate, fluoromethyl ketone or chloromethyl ketone functions are amongst the most common C-terminal modifications of peptides for generating effective inhibitors. The incorporation of such highly reactive moieties requires the adaptation of the synthetic strategy to each case, but our specialists can rely on their vast experience in SPPS and solution chemistry.

### O-Acylated Peptides

The peptide hormone ghrelin containing an O-acylated serine residue and its analogs have found widespread application in obesity research. Bachem gained considerable experience in performing this modification during the synthesis of many ghrelin analogs available in our catalog.

### Acid-Sensitive Modifications

Our chemists are able to fine-tune their synthetic tactics so precisely that even highly acid-sensitive peptides containing two or more sulfated tyrosines could be obtained.

### Stabilizing Modifications

A range of modifications for prolonging the half-life and increasing the metabolic stability of bioactive peptides can be performed, including selective PEGylation, incorporation of N-methylamino acids, and generation of pseudo-peptide bonds resisting enzymatic cleavage (e.g. reduced peptide bonds,  $\psi$ -[CH<sub>2</sub>-NH]).

### Peptides Containing Chelating Groups

Complexes formed between peptides bearing a chelating moiety such as DOTA or DTPA and radionuclides are increasingly used as imaging agents or for radionuclide delivery.

Bachem can rapidly deliver any peptide-chelator conjugates, but the complexing of the appropriate radionuclide has to be performed by the customer.

Peptides prone to aggregation such as the  $\beta$ -amyloid fragments require a special treatment. Ask Bachem for our technical brochure on handling and solubilization of  $\beta$ -amyloid peptides.

Ask Bachem for our technical documentation on handling and solubilization of peptides.

The derivatives of the chelators required for the coupling with the peptide are synthesized in-house.

Further examples of modified peptides and complex peptidomimetic compounds synthesized at Bachem could be added to this list. Feasibility studies for the synthesis of pseudopeptides containing hydroxy-ethylene or other isosteric bonds, peptide alcohols, decapeptides, or whatever you might conceive as your new chemical entity, can be conducted by our experts. We will be very pleased to send you a customized quotation.

### Care and Handling of Peptides

If stored under appropriate conditions peptides are rather stable. Nevertheless they should not be stored in solution (not even in sterile oxygen-free solution) as they may slowly chemically degrade.

Frozen solutions may be kept for a few weeks.

For longer storage, peptides should be kept as lyophilizate in a tightly closed container at  $< -15\text{ }^{\circ}\text{C}$ , the lower the temperature the better (long-term storage at  $-50\text{ }^{\circ}\text{C}$  or lower). Especially peptides containing Asn, Gln, Met, Cys, and/or Trp have limited shelf-lives. However, they may be shipped at room temperature, and for short-term use they may be stored in a refrigerator at  $4\text{ }^{\circ}\text{C}$ .

### Handling of Lyophilized Peptides

- Weigh out peptides quickly and reseal vial tightly.
- Peptides tend to be hygroscopic, therefore allow the vial to reach ambient temperature in a desiccator prior to opening and weighing out the peptide. Adsorption of water reduces the overall peptide content and may also decrease stability.

Please keep in mind to wear a dust respirator when handling larger amounts of peptide lyophilizates.

### Solubilization of Peptides

The reconstitution of a hydrophobic peptide may turn into an almost insurmountable obstacle in the course of your assay. As the

properties of peptides can vary extraordinarily, we can't offer you a standard protocol for dissolving a peptide. We can only offer you a large number of tips and tricks gained from our broad experience in handling these compounds.

The solubility of a peptide is determined mainly by its polarity. The amino acid composition will provide a first indication: before choosing a solvent, the sequence should be studied and the number of acidic, basic and neutral residues should be determined. The side-chain functionalities of acidic and basic amino acids will be charged at physiological pH. The nature of the N- and the C-terminal functionality has to be taken into consideration. The neutral amino acids can be roughly divided into two categories, non-polar, i.e. more or less hydrophobic residues and polar residues. "Dispersed" proline residues disrupt secondary structures and thus increase the solubility.

**Basic peptides** (number of basic amino acids including the N-terminal amino group larger than the number of acidic amino acids including the C-terminal carboxyl moiety) may be dissolved in a small amount of an acidic solvent such as acetic acid or trifluoroacetic acid and then diluted to the desired concentration. However, the safest diluent is PBS at pH 7.0 - 7.4 provided that a concentration of  $\leq 1\text{ mg/mL}$  is sufficient. If delivered as trifluoroacetates, peptides containing a relatively large proportion of Arg and Lys residues tend to be soluble at neutral pH.

**Acidic peptides** (number of acidic amino acids including the C-terminal carboxyl group larger than the number of basic amino acids including the N-terminal amino group) may be reconstituted in a small amount of a basic solvent such as 0.1% aqueous  $\text{NH}_3$  and then diluted with water to the desired concentration. However, as in the case of basic peptides, these may often be solubilized in PBS at pH 7.0 - 7.4 provided that a concentration of  $\leq 1\text{ mg/mL}$  is sufficient.

**Neutral or highly hydrophobic peptides** containing a high proportion of polar

Please note that peptides containing free cysteines should be dissolved in carefully degassed acidic buffers, as the thiol moieties will be rapidly oxidized at pH > 7 to disulfides.

uncharged amino acids and/or hydrophobic amino acids should be dissolved in a small amount of an organic solvent such as DMSO, DMF, acetic acid, acetonitrile, methanol, propanol, or isopropanol and then diluted with water (or buffer) to the desired concentration. Please keep in mind that high concentrations of these solvents are incompatible with biological systems such as cells.

Denaturing agents, such as urea or guanidinium hydrochloride may be used to solubilize peptides which tend to aggregate. As these additives interfere with most biological systems, their application is rather limited.

The reconstitution of a peptide may take time, occasionally up to several hours. Sonication for several minutes in a water bath may be helpful to accelerate the dissolution of larger particles. However, excessive warming of the sample should be avoided. Please note that peptides containing Trp, Met or Cys residues require special care to avoid oxidation. Oxygen-free water/buffers or reducing agents such as 1,4-dithio-DL-threitol (DTT) have to be used.

#### **Storage of Peptides in Solution**

- Dissolve peptides in an appropriate buffer (cf. Solubilization of peptides, p.14).
- For storage, peptide solutions should be aliquoted and kept frozen below -15 °C. Long-term storage of peptide solutions can't be recommended, especially when the peptide contains Asn, Gln, Cys, Met, or Trp.

You can download our Technical Notes and brochures from our homepage

<http://www.bachem.com/>

#### **Delivery Time**

The production of a custom peptide consists of four steps: synthesis, purification, lyophilization and analysis. Synthesis and purification are the most time-consuming steps.

#### **Synthesis**

Potential problems:

- Low yield
- Incomplete deprotection and coupling
- The peptide can't be obtained in acceptable purity by standard Fmoc/tBu-SPPS. Changing the type of carrier resin, or a different synthetic strategy may lead to the desired product
- Complete failure of synthesis

#### **HPLC Purification**

A low yield or complete loss of the peptide on the HPLC column may occur during the purification of hydrophobic peptides. Considerable difficulties have to be expected during the production of very hydrophobic long peptides and very short hydrophilic peptides. The production of acidic peptides containing free cysteines is one of the more precarious tasks, as during purification and handling in neutral or basic solution extreme caution is required to prevent the oligomerization of the peptide by disulfide bond formation.

Even if the production of a peptide seems rather straightforward, unexpected problems may occur. Hence we prefer to give you an approximate delivery time instead of a fixed term. Depending on the requested purity and amount of peptide, the delivery time varies from 10 days to several weeks.

# MOST FREQUENTLY ASKED QUESTIONS

Answers to frequently asked questions covering all topics concerning custom peptide synthesis can be found on the following pages.

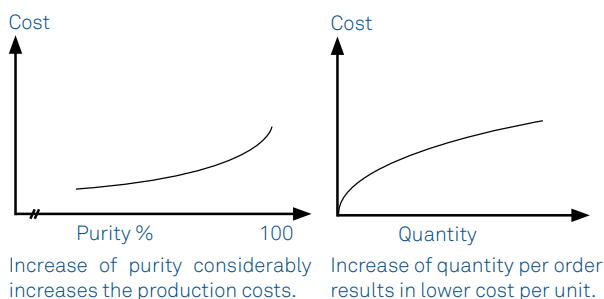


### Questions Related to the Calculation of Prices

Bachem adheres to a competitive price policy based on chart prices for each range of purity. For a given purity grade the price will depend on the sequence and the requested quantity.

#### What is the relationship between the production costs, the quantity, and purity requested?

The production costs of custom peptides depend markedly on the requested quantity and purity as described below :



### Questions Related to Quotation Inquiries and Orders

#### How should the peptide sequence be presented?

Starting from the N-terminal amino acid, please use the three letter code as standard. An unmodified N-terminus is depicted as H- (not NH<sub>2</sub>), a free C-terminus as -OH (not COOH). Modifications required on the lateral chain of amino acids are written in brackets following the abbreviation of the amino acid to be modified. If not explicitly denoted otherwise, all amino acids are introduced as L-enantiomers. If you require a more complex peptide, or if you have problems in producing an unambiguous presentation of your required structure, we are pleased to help you.

Examples:

N-terminal acetylation: Ac-Xaa-Yaa-...

A peptide amide: ...-Xaa-Yaa-NH<sub>2</sub>

A peptide carrying a biotinyl residue attached to the Lys side chain:

...Xaa-Lys(biotinyl)-Yaa ...

A head-to-tail cyclized peptide: (Xaa-...-Yaa)

A peptide containing a D-amino acid:

...-Xaa-D-Yaa-...

A peptide containing an N-methylated amino acid:

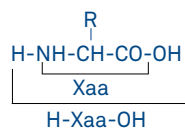
...-Xaa-N-Me-Yaa-... or ...-Xaa-MeYaa-...

If an unambiguous abbreviation for a moiety is lacking, please provide the full name, e.g. chloroacetyl.

#### What do H- on N-terminus and -OH on C-terminus signify?

H- : signifies a free N-terminus i.e. NH<sub>2</sub> or NH (Pro).

-OH : denotes a free C-terminus, a carboxylic acid (please do not omit the hyphens). The three letter code of an amino acid stands for NH-CHR-CO, i.e. H-Xaa-OH stands for H-NH-CHR-CO-OH.



If the N-terminus has to be acetylated, the H should be replaced by Ac.

When the C-terminus is amidated the OH has to be replaced by NH<sub>2</sub>. Replacement by H signifies a C-terminal aldehyde.

#### What kind of information should be given?

Additional information concerning the purity and quantity you require is most important.

Further material such as literature references would be highly welcome, especially if you are requesting the synthesis of an unusual peptide.

#### Should we specify the requested salt form?

If not specified otherwise, custom peptides are delivered as TFA salts. Usually, an additional step is required to generate another salt form, which will add to the price. Hence, we will quote for the TFA salt, if you don't explicitly request a different counter-ion such as the acetate or the hydrochloride. Due to deviating purification protocols, certain peptides (e.g. acidic peptides) will be delivered as ammonium salts.

**Do I have to order a minimal amount?  
What is the maximum quantity feasible at Bachem?**

The minimal quantity to be ordered depends on the requested purity, and is 5 mg for “Immunograde” peptides and 2 mg for the other grades of purity. There is no upper limit for the amount of research and GMP peptides. Our facilities enable us to produce peptides even up to ton-scale.

**Questions Related to the Synthesis**

**How do you synthesize peptides?**

Peptides are synthesized by chemical methods, either by solid-phase synthesis or by “classical” solution phase methods. The peptide is elongated starting from the C-terminus to the N-terminus of the sequence.

**What does SPPS mean?**

Solid-Phase Peptide Synthesis (SPPS) can be defined as the process in which a peptide is constructed by successive addition of the protected amino acids constituting its sequence, anchored via its C-terminus to an insoluble polymer.

For more information, please ask for your brochure *The Bachem Practice of SPPS*.

**What do Boc- and Fmoc-strategy mean?**

Two SPPS strategies have been developed, the Boc/Bzl and the Fmoc/tBu protection scheme. They differ in the types of protecting groups used for the free  $\alpha$ -amino group and the side-chain functionalities. Boc signifies t-butyloxycarbonyl, Bzl benzyl, tBu tert-butyl, and Fmoc stands for 9-fluorenylmethyloxycarbonyl. The Fmoc/tBu strategy is now considered standard procedure.

**What is the maximum peptide length feasible by chemical synthesis?**

Due to the continuing improvement of the methodology the maximum peptide length accessible by SPPS was steadily increased. It depends on the peptide sequence. Peptide comprising approximately 50 amino acids can be considered as the upper limit in routine SPPS. By adapting the synthetic protocols Bachem could obtain numerous peptides consisting of about 100 residues. Such molecules can be regarded as small proteins.

**How many free cysteines in a peptide are acceptable?**

4 to 5 free thiol moieties, depending on the amino acid composition of the peptide, is our upper limit. A replacement of Cys residues by less sensitive isosteric serines should always be considered, as these stable analogs will be obtained in higher purity. Peptides containing a single free thiol group may be oxidized yielding dimers. Cyclic peptides or oligomers may be obtained from peptides containing several Cys residues. Hence, we have to lower the purity on offer.

**How many disulfide bridges can be obtained?**

The maximal number of disulfide bridges depends on the method of disulfide bond formation. The consecutive formation of two to three disulfide bridges has to be considered as routine, a larger number requires sophisticated Cys protection schemes. The number of S-S bonds which can be generated simultaneously is not limited, either the peptide will fold into the required conformation in solution or the method does not work. Thus, peptides containing up to 5 disulfide bonds have been produced at Bachem.

**How many phosphorylated residues can be incorporated in a peptide?**

Two to three phosphorylated residues per peptide are a reasonable limit.

**What would be referred to as a standard modification?**

Standard modifications affect the final price of the peptide only slightly, as they can be easily integrated into a routine synthetic protocol. This includes acetylation, amidation, biotinylation, monophosphorylation, FITC labeling and similar modifications.

**What is the effect of N-terminal acetylation and C-terminal amidation?**

Acetylation and amidation reduce the overall charge of a peptide, thus the solubility may decrease. These modifications could increase the metabolic stability of the peptide as they prevent enzymatic degradation by exopeptidases. If the peptide comprises a partial sequence of a protein corresponding to an active site, the terminal acetylation/

amidation will generate a closer mimic of the native protein. Hence, this simple modification may increase the biological activity of a peptide considerably, not merely by prolonging its half-life.

**What do you recommend for avoiding pyroglutamate formation in case of an N-terminal Gln?**

Pyroglutamate formation can be prevented by N-terminal acetylation, incorporation of the preceding amino acid, or omission of the Gln (especially when synthesizing partial sequences). Even the acceptance of a certain extent of Pyr formation could be an option.

**Is a spacer required for introducing a fluorescent label?**

Most fluorescent dyes are large aromatic molecules. The incorporation of such bulky moieties may influence the biological activity of a peptide, an effect which can be alleviated by interposing a flexible spacer. On the other hand, the introduction of a spacer cannot be recommended when performing structural studies or when devising FRET substrates. In the latter case, the response could be modified. We strongly advise to search the literature for precedents when considering the incorporation of a linker.

**Do we have to expect batch-to-batch variability?**

Lot-to-lot variability will increase when producing low purity batches. However, even when obtaining very pure peptides ( $\geq 95\%$  or  $\geq 97\%$ ), the net peptide content can vary from batch to batch.

**Questions Related to Purity and Analytical Methods**

**What is an ADS?**

ADS stands for Analytical Data Sheet. It contains the following data:

- Lot number
- Type of product: catalog or custom-synthesized
- Product number
- Product description: name and sequence
- Molecular formula

- Relative molecular mass
- Tests performed, depending on the type of product and the purity requested, e.g.
  - Appearance
  - Molecular mass, obtained by mass spectrometry
  - Amino acid analysis
  - Purity (determined by HPLC)
  - N-content (determined by elemental analysis)
  - Solubility

**Where are the ADS available?**

The ADS can be downloaded from our website, [www.bachem.com](http://www.bachem.com). Please click on the link *Analytical Data Sheet (ADS)* on the welcome page of our web site. You have to enter the product number and the lot number, then click on *continue* to get your ADS as PDF file.

**Which analytical data do you provide?**

The kind of analytical data accompanying your product depends on the chosen purity range:

Grade of Purity	Data Obtained by
Immuno-grade	HPLC and MS
$\geq 80\%$	HPLC, MS, Net Peptide Content determination, AAA on request
90 - 95 %	HPLC, MS, Net Peptide Content determination, AAA on request
$\geq 95\%$	HPLC, MS, Net Peptide Content determination, AAA on request

**How do you purify the peptide?**

Purification of synthetic peptides is carried out by RP-HPLC and, in some cases, by ion exchange chromatography.

**What does HPLC purity mean?**

The purity determined by HPLC (%) corresponds to the percentage of requested peptide in relation to the total amount of material absorbing at 210 - 220 nm i.e. the desired product, peptidic by-products and other impurities.

**What does net peptide content mean?**

The net peptide content (NPC) is the fraction of peptidic material (i.e. the requested peptide and the peptidic impurities) relative to counter-ions and residual water. The latter do not contain nitrogen, allowing the determination of the net peptide content by elemental analysis. Additionally, it can be determined by AAA.

**What does gross weight mean?**

The gross weight of a peptide sample comprises the weight of the peptide, the salt (counter-ions) and the residual water.

Gross weight = Net weight/NPC

An example:

1 g (net) of peptide (NPC = 80%) is equivalent to 1.25 g (gross weight)

1g (net)/0.8 = 1.25 g (gross weight)

**What does immunograde mean?**

Immunograde peptides are peptides purified up to  $\geq 65\%$ . Their main use is in the production of polyclonal antibodies. At Bachem, the raw peptide obtained after cleavage from the resin is NEVER sold as "Immunograde" peptide even if it is sufficiently pure. It is ALWAYS subjected to preparative HPLC to remove the contaminants remaining from the cleavage and subsequent work-up.

**What are the remaining impurities?**

Not all impurities can be removed by a single HPLC run. Usually a few peptidic contaminants remain, mostly deletion sequences (peptides lacking one or more amino acids of the target sequence). Such by-products differ only slightly in polarity, hence their removal may fail. Truncation sequences (which may be generated deliberately by capping steps to avoid the formation of deletion peptides), incompletely deprotected peptides and by-products generated during the synthesis or during the final cleavage may also be found among the impurities.

**What kind of MS analyses are provided by Bachem?**

MALDI-TOF MS and/or ESI-MS, with 5 ppm mass accuracy.

**How to perform an amino acid analysis (AAA)?**

Amino acid analysis is performed by liquid-phase hydrolysis of the peptide with constant-boiling hydrochloric acid (6N HCl) followed by pre-column derivatization of the free amino acids with AccQ Fluor™ (Waters). The derivatized amino acids are separated by RP-HPLC using a C<sub>18</sub> 4 μm column. Integration of the individual peaks allows the determination of the amino acid composition of the peptide hydrolysate. However, the indole moiety of Trp is destroyed during hydrolysis; the side-chain amide groups of Asn and Gln and the lactam cycle of Pyr will be hydrolyzed yielding Asp and Glu, respectively. Hence, the AAA lists these amino acids as Asx and Glx.

**Questions Related to Handling and Storage****How to dissolve a peptide?**

Please refer to the section "Solubilization of Peptides" on p. 14 and see our Technical Note *Solubilization of Peptides*, which can be downloaded from our website

[www.bachem.com](http://www.bachem.com).

**Can I predict if a peptide is soluble in aqueous buffer?**

Unfortunately, the solubility of a peptide in water cannot be predicted just by studying the structure. However, a few clues can be deduced from the sequence: a relatively short peptide containing Lys and Arg residues will be soluble in aqueous buffers, especially as all basic functionalities will be protonated in peptides sold as trifluoroacetate salts. The guanidine function of Arg is a strong base, whereas the ε-amino group of Lys is a moderately strong base. By contrast, "acidic" peptides containing a large proportion of Asp and Glu tend to be insoluble in water, but they are readily dissolved by diluted ammonia and by basic buffers. The side-chain carboxy functions are rather weak acids, they are considerably less acidic than the C-terminal carboxyl group.

**How should peptides be stored ?**

For long-term storage the lyophilizate of the peptide should be kept in the deep freeze at  $< -15\text{ }^{\circ}\text{C}$ . For short-time storage a refrigerator ( $+4\text{ }^{\circ}\text{C}$ ) will suffice.

**How does Bachem ship peptides?  
Is dry ice required for the shipment?**

Dry ice is not required for the shipment of peptides (Express delivery) due to the high stability of the lyophilizates in sealed vials. Dry ice shipment will increase the price of your product considerably.

**Are custom peptides supplied as gross weight or as net peptide weight?**

Custom peptides are supplied as gross weight, unless requested otherwise.

**Are peptides containing free Cys supplied as monomers?**

The purity stated for peptides containing free Cys residues signifies the monomer content at the time the analytical HPLC was recorded. As air oxidation cannot be completely excluded in the meantime, we suggest reducing the peptide before use by treatment with dithiothreitol (DTT). For a short description of the cystine reduction using DTT please see our Technical Note *Reversal of Inadvertent Oxidation of Cys-containing Peptides* which can be downloaded from our homepage.

**Could you provide special packing?**

Special packaging and vialing are available upon request and will be charged additionally.

**How long will delivery take?**

Even if the production of a peptide seems rather straightforward, unexpected problems may occur. Hence we prefer to give you an approximate delivery time window instead of a fixed term. Depending on the requested purity, chemical complexity, and quantity of your peptide, typical delivery times range from 3-4 weeks to 2-3 months. In urgent matters please ask for our Express delivery service. Note: The requested peptide could already be offered as catalog product in our online shop comprising more than 6.500 items. If this is the case, it can be delivered within 1-2 working days.

**Conclusion**

Why choose Bachem for your custom synthesis?

Our technological leadership position is based on more than forty years experience in peptide manufacturing. From the outset, various new pathways of synthesis are evaluated and compared in terms of their efficiency. As part of our service, we provide technical consultancy on the synthetic feasibility, stability, and solubility of your compounds. We offer suggestions to improve proposed molecular structures that will simplify manufacture or increase stability. Bachem has the largest production capacity with four production sites in Switzerland, UK, and the USA. Automatic and semi-automatic synthesizers in all sizes and adequate equipment for purification and lyophilization are available for synthesizing your peptide in mg- to ton-scale. Reactors of all sizes are available for the synthesis of complex organic compounds. We can produce a simple research peptide in mg-scale as well as the most complex peptidomimetic in kg- to ton-scale. Additionally, we can produce your active compound under cGMP-conditions including the preparation of the required documentation.

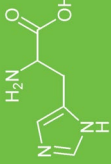

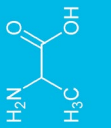
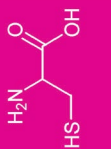
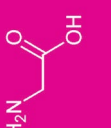


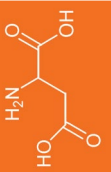

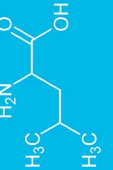
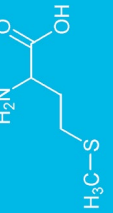


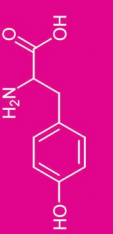
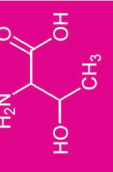
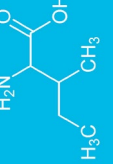

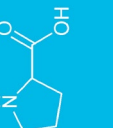
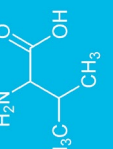
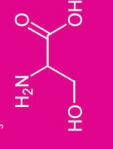
We offer high flexibility, reliable quality, competitive prices, and short delivery times. Bachem is your preferred service provider for your custom synthesis and catalog peptides.

**Abbreviations**

AAA	Amino Acid Analysis	Gal	Galactosyl
Aad	$\alpha$ -Aminoadipyl	Glc	Glucosyl
Abu	$\alpha$ -Aminobutyryl	Glx	Gln or Glu or Pyr (AAA)
ADS	Analytical Data Sheet	GMP	Good Manufacturing Practice
AEEAc	2-[(2-Amino)ethoxy]ethoxyacetic acid	HPLC	High Performance Liquid Chromatography
AMC	7-Amido-4-methylcoumarin	KLH	Keyhole Limpet Hemocyanin
Asu	$\alpha$ -Aminosuberyl	MS	Mass Spectrometry
Asx	Asn or Asp (AAA)	Nle	Norleucyl
Boc	t-Butyloxycarbonyl	NPC	Net Peptide Content
BSA	Bovine Serum Albumin	Orn	Ornithyl
Bzl	Benzyl	OVA	Ovalbumin
cGMP	Current Good Manufacturing Practice	Pam	Palmitoyl
Dab	$\alpha,\gamma$ -Diaminobutyryl	PBS	Phosphate Buffered Saline
Dap	$\alpha,\beta$ -Diaminopropionyl	PEG	Polyethylene Glycol
DMF	Dimethylformamide	pNA	4-Nitroanilide
DMSO	Dimethylsulfoxide	Pyr	Pyroglutamyl
DOTA	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid	RP-HPLC	Reversed Phase High Performance Liquid Chromatography
DPTA	Diethylenetriaminepentaacetic acid	SPPS	Solid-Phase Peptide Synthesis
DTT	1,4-Dithio-DL-threitol	tBu	t-Butyl
FITC	Fluorescein isothiocyanate	TFA	Trifluoroacetic acid
Fmoc	9-Fluorenylmethyloxycarbonyl	Xaa,	Unspecified $\alpha$ -amino acids
FRET	Fluorescence Resonance Energy Transfer	Yaa	

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PIONEERING PARTNER FOR PEPTIDES

<b>H</b> 155.16 137.14 $C_6H_9N_3O_2$  <b>His</b> Histidine	<b>F</b> 165.19 147.18 $C_9H_9NO_2$  <b>Phe</b> Phenylalanine	<b>A</b> 89.09 71.08 $C_3H_7NO_2$  <b>Ala</b> Alanine	<b>C</b> 121.16 103.14 $C_3H_7NO_2S$  <b>Cys</b> Cysteine	<b>G</b> 75.07 57.05 $C_2H_5NO_2$  <b>Gly</b> Glycine	<b>Q</b> 146.15 128.13 $C_5H_9N_2O_3$  <b>Gln</b> Glutamine	<b>E</b> 147.13 129.11 $C_5H_9NO_4$  <b>Glu</b> Glutamic Acid	<b>D</b> 133.10 115.09 $C_4H_7NO_4$  <b>Asp</b> Aspartic Acid				
<b>K</b> 146.19 128.17 $C_6H_{13}N_3O_2$  <b>Lys</b> Lysine	<b>L</b> 131.18 113.16 $C_6H_{13}NO_2$  <b>Leu</b> Leucine	<b>M</b> 149.21 131.20 $C_5H_{11}NO_2S$  <b>Met</b> Methionine	<b>N</b> 132.12 114.10 $C_4H_8N_2O_3$  <b>Asn</b> Asparagine	<b>S</b> 105.09 87.08 $C_3H_7NO_3$  <b>Ser</b> Serine	<b>Y</b> 181.19 163.17 $C_9H_9NO_3$  <b>Tyr</b> Tyrosine	<b>T</b> 119.12 101.10 $C_4H_9NO_3$  <b>Thr</b> Threonine	<b>I</b> 131.18 113.16 $C_6H_{13}NO_2$  <b>Ile</b> Isoleucine	<b>W</b> 204.23 186.21 $C_{11}H_{12}N_2O_2$  <b>Trp</b> Tryptophan	<b>P</b> 115.13 97.12 $C_5H_9NO_2$  <b>Pro</b> Proline	<b>V</b> 117.15 99.13 $C_6H_{11}NO_2$  <b>Val</b> Valine	<b>Ser</b> Serine Chemical Name  Chemical Structure $C_3H_7NO_3$ Molecular Formula $M_r - H_2O$ 87.08 Relative Molecular Mass 105.09 1-Letter Amino Acid Code <b>S</b> 3-Letter Amino Acid Code <b>Ser</b>

BACHEM

LEADING PARTNER IN TIDES

## Periodic Chart of Amino Acids

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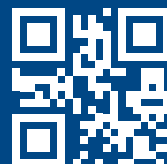
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