ISOTOPE-LABELED PEPTIDES

BACHEM

PIONEERING PARTNER FOR PEPTIDES
In addition to our research-grade peptides and generic API products Bachem offers deuterated, $^{13}\text{C}$-, and $^{15}\text{N}$-labeled analogs for pharmacokinetic/pharmacodynamic studies as well as analogs suitable for radiolabeling. Contrary to working with radionuclide-labeled peptides, licenses and additional safety measures are not required for handling stable isotope-labeled peptides.

In recent times, there have been dramatic improvements in the identification and development of novel biomarkers for diagnostic and therapeutic applications. The advent of stable isotopes has allowed the relative or even absolute quantification of proteins by mass spectrometric techniques based upon their well-defined increase in molecular mass compared to the native protein or peptide of interest. When combined with the masses of data continuously being generated in proteomics, such quantification can be used in the detection and development of innovative biomarkers. This approach can be particularly valuable when trying to compare and contrast the levels of specific proteins in two different biological states, like in normal and pathophysiological cells or cells before and after drug treatment.

**Heavy Isotope-labeled Peptides in Mass Spectrometry**

Biological molecules such as proteins mainly consist of carbon, hydrogen, nitrogen, and oxygen. None of these elements is monoisotopic, they exist as stable and radioactive isotopes, i.e. atoms with differing atomic weight. $^{13}\text{C}$ has the same number of protons and electrons as $^{12}\text{C}$, so it behaves chemically in the same manner, forming the same types of chemical bonds. However, its nucleus contains an additional neutron, making it heavier. The same goes for deuterium ($^2\text{H}$ or D), and $^{15}\text{N}$; $^{18}\text{O}$ consists of 8 protons and 10 neutrons. $^2\text{H}$, $^{13}\text{C}$, and $^{15}\text{N}$ naturally occur at only about 1% abundance, $^{18}\text{O}$ merely 0.2%.

Amino acids containing such atoms can be incorporated into peptides during synthesis (Fig. 1). The resulting “weight gain” (Fig. 2) can be exploited in various ways, most notably as internal standards for protein quantification by mass spectrometry. Mass spectrometry (MS) works by ionizing compounds and measuring the mass-to-charge ratio of the generated molecule ions.

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**ISOTOPE LABELING OF PEPTIDES**

The use of radionuclide- and stable isotope-labeled peptides enhances drug discovery and development. Besides non-radioactive isotope-labeled amino acid derivatives and peptides Bachem offers analogs of bioactive peptides suitable for radioiodination or tritiation. Only tyrosine-containing peptides or peptides modified with Bolton-Hunter reagent can be radiolabeled directly.
and their charged fragments. MS has been an invaluable tool in proteome analysis. Yet the quantitative detection of clinically important proteins, like biomarkers or allergens, that are present in only minute quantities in a complex mixture like a cell lysate, or proteins that have been posttranslationally modified, has been challenging. Stable isotope-labeled peptides have successfully been used as internal standards in MS to provide absolute protein quantification for at least forty years (1), and their utility has only increased as technological developments in mass spectrometry have broadened their applicability in proteomic studies. Generally, the ratio of peak intensities of the “light” native peptide and “heavy” isotopic peptide is used to calculate the relative protein abundance. This approach permits the simultaneous evaluation of numerous proteins from defined biological states.

**Labeled Tags and Linkers for Relative Quantification**

In techniques such as ICAT (Isotope Coded Affinity Tags) and iTRAQ (isobaric Tags for Relative and Absolute Quantification), peptides that are generated by proteolytic digests of cellular lysates are covalently bound to isotopically labeled tags that have different masses. In ICAT an isotopically coded linker is used to attach the proteolytic products to a tag such as biotin, that can be subsequently used for purification (2). For quantitative comparison of two proteomes by ICAT, a sample is labeled with the isotopically light probe and a second one with the heavy-isotope version. After proteolytic digestion, the labeled peptides are analyzed by liquid chromatography-mass spectrometry (LC-MS), and the ratios of the signal intensities of the differentially mass-tagged peptide pairs are quantified to determine the relative levels of proteins in the two samples.

**Figure 1:** Examples of stable isotope-labeled tripeptides:
A) Glutathion containing a $^{13}$C,$^{15}$N-labeled glycine (M + 3),
B) FPR containing ring-deuterated phenylalanine and an arginine modified by a $^{15}$N-labeled guanidino moiety (M + 8).

**Figure 2:** ESI-mass spectra of
A) angiotensin II, MH$^+$ 1046.53,
B) ([ring-$D_5$]Phe$^*_8$)-angiotensin II, MH$^+$ 1051.57 / MNa$^+$ 1073.55 / MK$^+$ 1089.52.
In iTRAQ, the proteolytic products are labeled with one of either four or eight (depending on the experimental design) isobaric reagents (3). This enables the simultaneous identification and quantitation of proteins in different samples using tandem mass spectrometry. During the MS/MS analysis, each isobaric tag produces a unique reporter ion signature that allows for quantification. Although the labeled peptides are indistinguishable from each other in the first MS analysis because they do not differ in mass, each tag generates a unique reporter ion in the tandem MS mode when peptides are isolated and fragmented. Comparing the intensities of the different reporter ions in the MS/MS spectra yields data on the relative amounts of the labeled peptides.

**Labeled Peptides for Absolute Quantification**

Other methods, like MRM (Multiple Reaction Monitoring) and AQUA (Absolute QUAntification of proteins), rely on peptides containing heavy isotopes rather than linkers or tags. The labeled peptides are chosen to mimic proteolytic fragments of a protein to be measured, and then used as quantitative internal standards. MRM has been used to detect and quantify low abundance proteins in plasma, and can thus be harnessed in biomarker analysis (4). It requires the synthesis of a stable isotope-labeled peptide chemically identical to one the peptides generated by the tryptic digestion of the protein to be measured. A known quantity of this labeled peptide is used as an internal standard against which the chosen tryptic peptide can be quantified. C-reactive protein, apolipoprotein A-1, human growth hormone, and prostate-specific antigen have been measured in serum using this approach (5).

AQUA has been used to quantify low abundance proteins in yeast and to quantitatively determine the percentage of human separase protein phosphorylated in a cell cycle-dependent manner (5). First the labeled peptide is analyzed by MS/MS to establish the fragmentation patterns, and then the abundance of a specific fragment ion from both the native tryptic peptide and the stable isotope-labeled synthetic peptide are measured as a function of HPLC retention time. The absolute amount of the native peptide is determined by comparing its retention time to that of the known quantity of the labeled peptide.

**IR- and NMR-Spectroscopy**

Heavy isotope labeling is a valuable tool for determining the conformation of peptides by spectroscopic methods. Isotope exchange shifts the absorption frequencies of covalent bonds, so it allows conformational studies by infrared spectroscopy.

Nuclear Magnetic Resonance (NMR) is a powerful technique for determining the structures, dynamics, and molecular interactions of biomolecules. As more and more peptides advance in clinical trials, both as therapeutic agents and as vaccines, NMR can be used to measure their relaxation rates as they dissociate from their bound target (6). And since peptides often retain biological activity, they can stand in for whole proteins to simplify structural studies. NMR signals can only be obtained with isotopes with an uneven number of protons and/or neutrons. Such isotopes have a nonzero nuclear spin and thus absorb radiofrequency waves in a strong magnetic field causing a flip of the spin. Non-labeled peptides can be studied by \(^1\)H and \(^{13}\)C-NMR (detecting the naturally occurring amount of the isotope, 1.1%). Peptides labeled with \(^1\)H (spin 1), \(^{13}\)C (spin 1/2), and \(^{15}\)N (spin 1/2) are used especially in heteronuclear NMR studies (6).

Stable isotope-labeled peptides will continue to find numerous applications in drug research and development.

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G. Tőth et al.
Radiotracers, tritium labelling of neuropeptides.
ISOTOPE-Labeled Peptides

For our offer of heavy isotope labeled peptides and amino acid derivatives please see the next pages. If you require a different labeling scheme or labeled peptides not included in the list, our custom synthesis service is at your disposal. Please ask for a quote.
The list also includes peptides and amino acid derivatives for radiolabeling.
If the precursor peptide you require isn’t available, we would be pleased to offer you a custom synthesis. Please keep in mind that we don’t produce radiolabeled peptides.
For further information on our offer of peptides and building blocks please visit our online shop at shop.bachem.com
## Isotope Labeled Peptides

### HEAVY ISOTOPE-LABELED PEPTIDES AND THEIR NATIVE COUNTER-PARTS

<table>
<thead>
<tr>
<th>Isotope Labeled Peptide</th>
<th>Formula</th>
<th>Native Counter-Part</th>
</tr>
</thead>
<tbody>
<tr>
<td>([ring-D₅]Phe⁸)-Angiotensin II</td>
<td>H-7256</td>
<td>Angiotensin II (H-1705)</td>
</tr>
<tr>
<td>([¹³C]Leu¹⁰)-CRF (human, rat)</td>
<td>(((¹³C]Leu¹⁰)-Corticorelin)</td>
<td>CRF (human, rat) (Corticorelin) (H-2435)</td>
</tr>
<tr>
<td>([D₇]Val¹⁷⁻¹⁰)-C-Peptide (human)</td>
<td>H-4242</td>
<td>C-Peptide (human) (H-2470)</td>
</tr>
<tr>
<td>([¹³C]Leuⁿ⁻)-Endothelin-1 (human, bovine, dog, mouse, porcine, rat)</td>
<td>H-7254</td>
<td>Endothelin-1 (human, bovine, dog, mouse, porcine, rat) (H-6995)</td>
</tr>
<tr>
<td>([¹³C]Leuⁿ⁻)-Ghrelin (human)</td>
<td>H-7252</td>
<td>Ghrelin (human) (H-4864)</td>
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<tr>
<td>([¹⁴N]Gly)-Glutathione</td>
<td>H-4586</td>
<td>Bachem doesn't offer glutathione, but a selection of glutathione-related peptides</td>
</tr>
<tr>
<td>([¹⁴N]Gly)-Glutathione (reduced)</td>
<td>H-4584</td>
<td></td>
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<tr>
<td>(Des-Gly¹⁰,D-Leu⁶,[¹³C]Leu⁷,Pro-NHET⁹)-LHRH</td>
<td>H-6258</td>
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<tr>
<td>([ring-D₅]Phe⁹)-Octreotide</td>
<td>H-7238</td>
<td>Octreotide (H-5972)</td>
</tr>
<tr>
<td>([¹⁴C]₈,¹⁵N]Leu³⁻¹⁸,²⁰,³¹,³³)-Orexin A (human, mouse, rat) NEW</td>
<td>H-8336</td>
<td>Orexin A (human, mouse, rat) (H-4172)</td>
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<tr>
<td>([D₅]Gly⁴)-Cholecystokinin Octapeptide (sulfated)</td>
<td>H-7248</td>
<td>Cholecystokinin Octapeptide (sulfated) (Sincalide, CCK-8) (H-2080)</td>
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<tr>
<td>([ring-D₅]Phe⁹)-Somatostatin-14</td>
<td>H-7246</td>
<td>Somatostatin-14 (H-1490)</td>
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<tr>
<td>([¹⁴C]⁹⁻)-Thymosin β₄ (human, bovine, horse, rat)</td>
<td>H-7244</td>
<td>Thymosin β₄ (human, bovine, horse, rat) (H-2608)</td>
</tr>
</tbody>
</table>
**2H- AND 15N-Labeled Amino Acids**

- Fmoc-[D₄]Ala-OH  B-4130
- Fmoc-[D₂]Gly-OH  B-4125
- Fmoc-[D₁₀]Leu-OH  B-4120
- Fmoc-[ring-D₅]Phe-OH  B-4115
- Fmoc-[¹⁵N]Leu-OH  B-2655
- H-¹⁵N]Tyr-OH  E-3245

**Tyr-PEPTIDES FOR RADIOIODINATION**

- Tyr-Amloid P Component (27-38) amide  H-2944
- (Tyr²⁰)-Atroiopeptin II (rat)  H-2120
- (Tyr²⁰)-Apelin-13 (human, bovine, mouse, rat)  H-4894
- (Tyr²⁰)-BNP-32 (human)  H-5698
- Tyr-Bradykinin  H-2195
- (Tyr²⁰)-Bradykinin  H-1975
- (Tyr²⁰)-C-Peptide (human)  H-4934
- Tyr-α-CGRP (human)  H-3354
- Tyr-α-CGRP (23-37) (mouse, rat)  H-2270
- (Tyr²⁰)-a-CGRP (27-37) (canine, mouse, rat)  H-5504
- (Tyr²⁰)-Cholecystokinin Octapeptide (sulfated)  H-9770
- Tyr-CRF (human, rat)  H-2455
- (Tyr²⁰)-Fibrinopeptide A (human)  H-2945
- (Tyr¹⁹)-Fibrinopeptide B (human)  H-2955
- Tyr-Leptin (26-39) (human)  H-3494
- Tyr-LL-37  NEW  H-7902
- (Phe¹³,Tyr¹⁹)-MCH (human, mouse, rat)  H-2218
- (Tyr²⁰)-Melanocyste-Stimulating Hormone-Release Inhibiting Factor (Tyr-MIF-1)  H-5120
- (Tyr²⁰)-Neurokinin A  H-9270
- Tyr-PDGF A-Chain (194-211)  H-8335
- (Tyr₀)-Prepro-Atrial Natriuretic Factor (104-123) (human)  H-5516
- Tyr-Proinsulin C-Peptide (55-89) (human)  H-2465
<table>
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<th><strong>Tyr-PEPTIDES FOR RADIO-IODINATION (CONT)</strong></th>
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<tr>
<td><em>(Tyr&lt;sup&gt;1&lt;/sup&gt;)-pTH (1-34) (human)</em></td>
<td><em>Tyr-Uroguanylin (mouse, rat)</em></td>
</tr>
<tr>
<td>H-3092</td>
<td>H-4148</td>
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<tr>
<td>*(Tyr&lt;sup&gt;42&lt;/sup&gt;)-pTH (7-34) amide (bovine)</td>
<td><em>(Phenylac&lt;sup&gt;1&lt;/sup&gt;,D-Tyr&lt;sup&gt;2&lt;/sup&gt;(Me)&lt;sup&gt;2&lt;/sup&gt;,Arg&lt;sup&gt;8&lt;/sup&gt;,&lt;sup&gt;8&lt;/sup&gt;Tyr-NH$_2$)&lt;sup&gt;2&lt;/sup&gt;</em>-Vasopressin*</td>
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<tr>
<td>N-1110</td>
<td>H-3194</td>
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<tr>
<td><strong>Tyr&lt;sup&gt;36&lt;/sup&gt;</strong>-pTH-Related Protein (1-36)</td>
<td><em>(d(CH$_2$)$_2$,&lt;sup&gt;1&lt;/sup&gt;Tyr(Me)&lt;sup&gt;2&lt;/sup&gt;,Thr&lt;sup&gt;4&lt;/sup&gt;,Orn&lt;sup&gt;8&lt;/sup&gt;,Tyr-NH$_2$)&lt;sup&gt;2&lt;/sup&gt;</em>-Vasotocin*</td>
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<tr>
<td><em>(human, mouse, rat)</em></td>
<td>H-9405</td>
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<td>H-3208</td>
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<tr>
<td>*(Tyr&lt;sup&gt;36&lt;/sup&gt;)-pTH-Related Protein (1-36) amide</td>
<td>For-Nle-Leu-Phe-Nle-Tyr-Lys-OH</td>
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<tr>
<td><em>(chicken)</em></td>
<td><em>(fMLF analog)</em></td>
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<td>H-5496</td>
<td>H-3065</td>
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<tr>
<td><strong>Tyr-Somatostatin-14</strong></td>
<td><strong>Cyclo(-Arg-Gly-Asp-D-Tyr-Lys)</strong></td>
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<td>H-4995</td>
<td>NEW (c(RGDyK))</td>
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<td><strong>Tyr-Somatostatin-28</strong></td>
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<td>H-4990</td>
<td><strong>Cyclo(-Arg-Ala-Asp-D-Tyr-Lys)</strong></td>
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<tr>
<td>*(Leu&lt;sup&gt;8&lt;/sup&gt;,D-Trp&lt;sup&gt;22&lt;/sup&gt;,Tyr&lt;sup&gt;25&lt;/sup&gt;)-Somatostatin-28</td>
<td>NEW (c(RADyK))</td>
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<td>H-3202</td>
<td>H-8144</td>
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<tr>
<td><em>(Tyr&lt;sup&gt;7&lt;/sup&gt;)-Stresscopin (human)</em></td>
<td><strong>H-Tyr-Arg-Gly-Asp-Ser-OH</strong></td>
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<td>H-5842</td>
<td><em>(YRGDS)</em></td>
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<td><em>(Tyr&lt;sup&gt;7&lt;/sup&gt;)-Substance P</em></td>
<td>H-3154</td>
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<td>H-1915</td>
<td><strong>H-Tyr-Gln-Ser-Leu-Arg-Trp-NH$_2$</strong></td>
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<tr>
<td><em>(Tyr&lt;sup&gt;7&lt;/sup&gt;)-TRAP-7</em></td>
<td><em>(Tyr&lt;sup&gt;2&lt;/sup&gt;,Gln&lt;sup&gt;1&lt;/sup&gt;)-Antho-RWamide I</em></td>
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<tr>
<td><em>(Tyr&lt;sup&gt;7&lt;/sup&gt;)-PAR-1 (1-7) (human)</em></td>
<td>H-5255</td>
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<td>H-1674</td>
<td><strong>H-D-Tyr-Pro-Arg-chloromethylketone</strong></td>
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<tr>
<td><em>(Tyr&lt;sup&gt;7&lt;/sup&gt;)-Urocortin (rat)</em></td>
<td><em>(PPACK analog)</em></td>
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<td>H-5486</td>
<td>N-1225</td>
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</tbody>
</table>
# Peptides for Tritiation

- **(3,5-Diiodo-Tyr¹,D-Ala²,N-Me-Phe³,glycinol⁴)-Enkephalin**
  - ((3,5-Diiodo-Tyr¹)-DAMGO)
  - H-2595

- **(3,5-Diiodo-Tyr⁵)-LHRH**
  - H-1375

- **For-Met-Leu-p-iodo-Phe-OH**
  - (fMLF analog)
  - H-3025

# Amino Acid Derivatives for Tritiation

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<tr>
<th>Fmoc-4,5-dehydro-Leu-OH</th>
<th>Boc-4,5-dehydro-Leu-OH · DCHA</th>
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<td>A-3485</td>
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<th>Boc-3,4-dehydro-Pro-OH</th>
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<td>B-1660</td>
<td>A-1550</td>
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<th>Fmoc-p-iodo-Phe-OH</th>
<th>Boc-p-iodo-Phe-OH</th>
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<td>B-2750</td>
<td>A-1800</td>
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<th>Boc-p-iodo-D-Phe-OH</th>
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<td>A-3640</td>
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<table>
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<tr>
<th>Fmoc-3,5-diiodo-Tyr-OH</th>
<th>Boc-3,5-diiodo-Tyr-OH</th>
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<td>B-2185</td>
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<th>Fmoc-Pra-OH</th>
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<th>Fmoc-D-Pra-OH</th>
<th>Boc-Pra-OH</th>
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<td>B-4150</td>
<td>A-4735</td>
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<table>
<thead>
<tr>
<th>Fmoc-Pra-Wang resin (200-400 mesh)</th>
<th>H-2,5-Diiodo-His-OH · HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-2820</td>
<td>F-3460</td>
</tr>
</tbody>
</table>
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