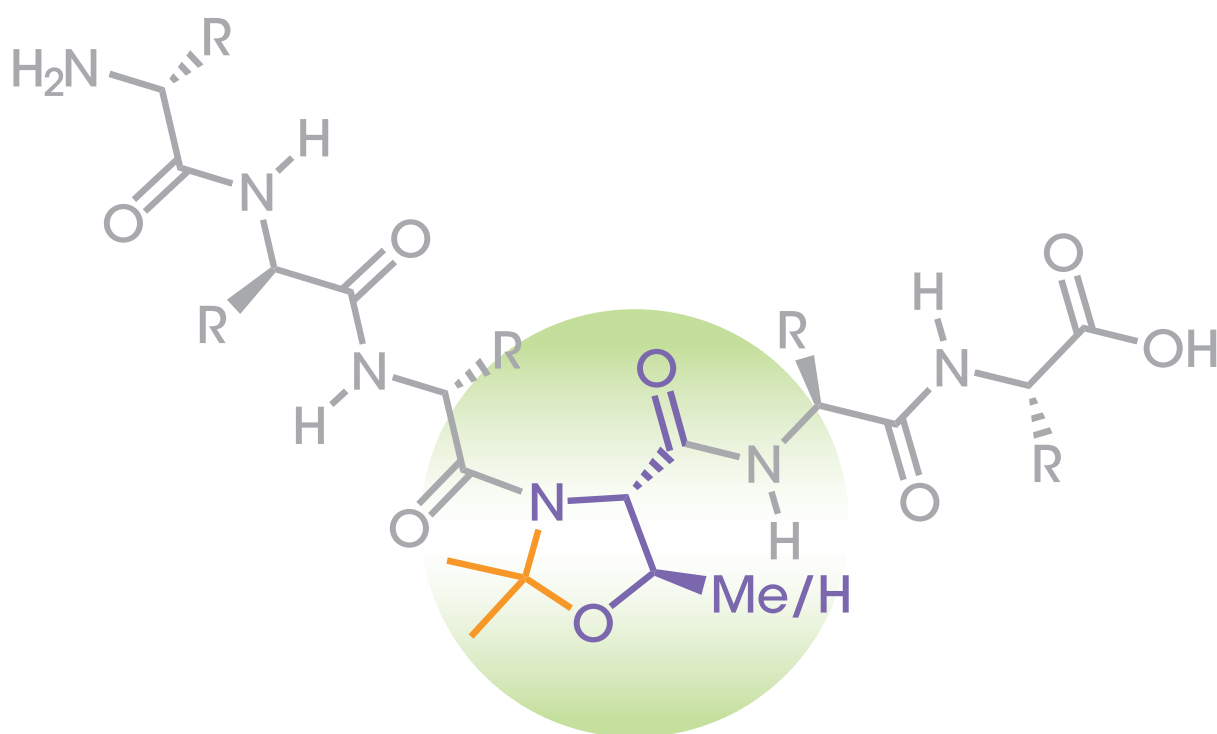


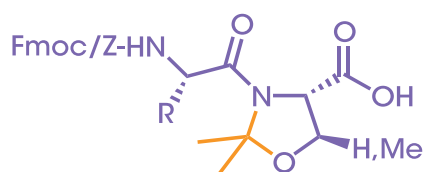
Genzyme Pharmaceuticals

PSEUDOPROLINE DIPEPTIDES

FOR MORE COST-EFFECTIVE LARGE-SCALE PRODUCTION OF PEPTIDES



GENZYME
PHARMACEUTICALS
NOW OFFERS
PSEUDOPROLINE
DIPEPTIDES FOR GMP
MANUFACTURING
OF PEPTIDES



Pseudoprolines are introduced as dipeptide building blocks to peptide synthesis.

Genzyme Pharmaceuticals continually strives to reduce the production cost of peptide APIs through manufacturing efficiencies and low raw material prices. With in-house multi-ton production capabilities and extensive experience in manufacturing a wide range of chiral building blocks, natural and non-natural amino acid derivatives, Genzyme Pharmaceuticals provides the economies of scale for a reliable and sustainable supply of key raw materials with consistent quality and highly competitive manufacturing costs.

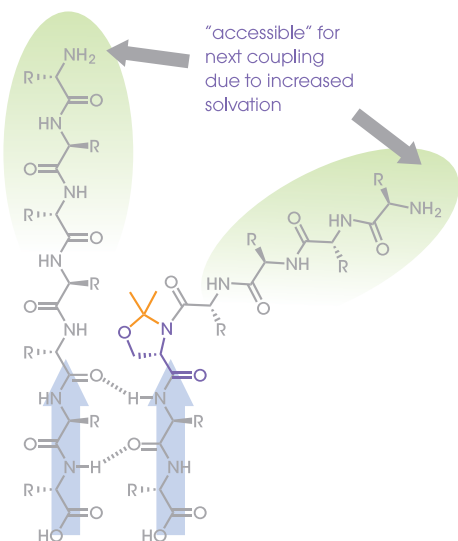
API production costs remain relatively high due to low yields of “difficult” peptide sequences and decreasing yields with increasing peptide length. Low solvation during solid phase peptide synthesis (SPPS) leads to peptide chain aggregation, typically accompanied by the formation of β -sheet structures¹. Low solubility of fully protected peptide segments is also a common limitation in process development and an important cost factor in peptide API production.

Advantages of Pseudoproline Dipeptides

Mutter’s serine and threonine derived pseudoproline dipeptides² have been shown to be a powerful tool for bypassing the synthesis challenges associated with “difficult sequences” and long peptides³ such as low solvation during solid phase synthesis, and limited solubility of (un)protected peptide segments.

Pseudoproline dipeptides may be incorporated into most common peptide synthesis strategies because:

- They can be coupled to growing peptide chains using standard procedures and coupling reagents
- They are easily cleaved with standard TFA mixtures
- They are compatible with synthesis strategies involving Fmoc- and Z-amino acids



Due to the presence of the proline-like ring system (fixed Φ -angle), in addition to the preference for a cis-amide bond with the preceding residue,⁴ the incorporation of a pseudoproline moiety exerts a pronounced effect upon the peptide backbone, thus preventing peptide aggregation, self-association or β -structure formation.

1 M. Mutter, S. Vuilleumier, *Angew. Chem. Int. Ed. Engl.*, (1985) 28, 535.

2 a) M. Mutter, A. Nefzi, T. Sato, F. Wahl, T. Wöhr, *Peptide Res.*, (1995) 8, 145-153; b) T. Wöhr, B. Rohwedder, F. Wahl, A. Nefzi, T. Sato, X. Sun, M. Mutter, *J. Am. Chem. Soc.*, (1996) 118, 9218-9227.

3 e.g.: a) W.R. Sampson, H. Patsiouras, N.J. Ede, *J. of Peptide Science*, (1999) 5, 403-409; b) A. Abedini, D.P. Raleigh, *Organic Letters*, (2005) 7, 693-696.

4 P. Dumy, M. Keller, D.E. Ryan, B. Rohwedder, T. Wöhr, M. Mutter, *J. Am. Chem. Soc.*, (1997) 119, 918-925.

Segment Condensation

Ser and Thr derived pseudoproline dipeptides offer additional segmentation sites for convergent solution/solid phase synthesis strategies. C-terminal pseudoproline dipeptides also guarantee epimerization-free segment condensation as their ring systems protect Ser and Thr from stereomutation via the oxazolone route.

Cyclic Peptides

Incorporation of a pseudoproline dipeptide into a peptide backbone directs the amide bond to the preceding residue, preferably into a *cis* conformation.⁴ A centrally placed pseudoproline dipeptide can therefore improve the peptide cyclization kinetics by aligning the disulfide bridge forming Cys side chains. The resulting crude cyclic peptide is of higher purity, thereby simplifying HPLC purification.

Large-scale Peptide Production

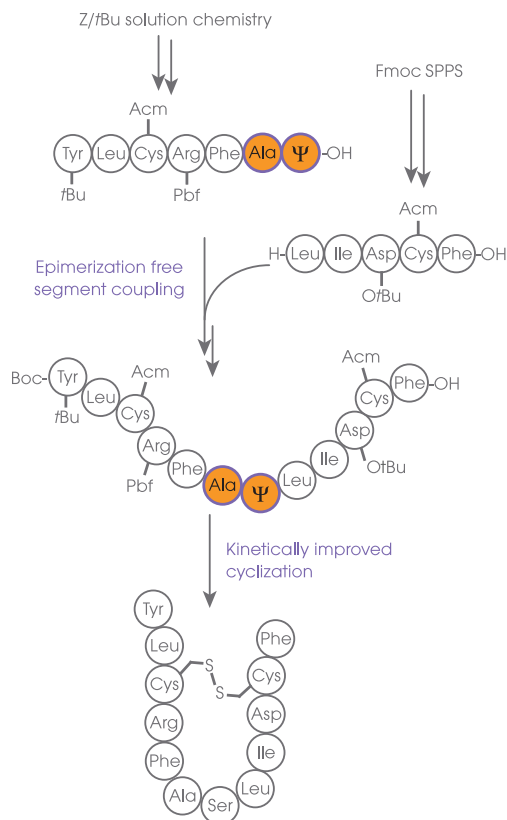
Pseudoproline dipeptides have important advantages for large-scale peptide production:

- Greatly improved chain elongation for hydrophobic and other “difficult” sequences
- Higher purity of crude peptide, thereby simplifying preparative HPLC purification
- Improved overall synthesis yields

GENZYME PHARMACEUTICALS NOW PROVIDES AN INCREASING RANGE OF PSEUDOPROLINE DIPEPTIDES PRACTICAL FOR LARGE-SCALE PRODUCTION

- Fmoc- or Z-protected pseudoproline dipeptides
- Quantities from 100g to hundreds of kilograms
- 10g and 25g packs also available
- High purity (well above 95%)

UNTIL NOW, LIMITED AVAILABILITY OF ECONOMICALLY PRICED PSEUDOPROLINE DIPEPTIDES IN BULK QUANTITIES WITH CONSISTENTLY HIGH PURITY HAS RESTRICTED THEIR WIDESPREAD USE, PARTICULARLY IN LARGE-SCALE PEPTIDE PRODUCTION.



Examples of readily available pseudoproline dipeptides are shown below. Others are available upon request.

Fmoc-Gly-Ser($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Gly-Thr($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Ala-Ser($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Ala-Thr($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Phe-Thr($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Phe-Ser($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Leu-Thr($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Leu-Ser($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Val-Ser($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Val-Thr($\Psi^{\text{Me,Me}}$ pro)-OH

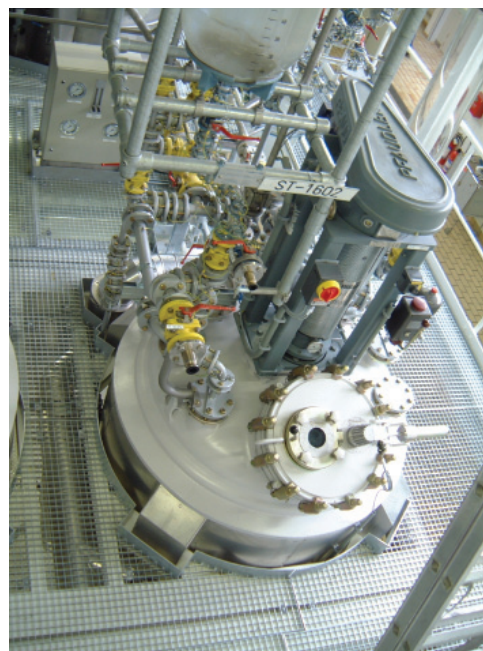
Fmoc-Ser(tBu)-Ser($\Psi^{\text{Me,Me}}$ pro)-OH

Fmoc-Ser(tBu)-Thr($\Psi^{\text{Me,Me}}$ pro)-OH

CONTACT GENZYME PHARMACEUTICALS FOR PSEUDOPROLINE DIPEPTIDES

With experience in all stages of method development, scale-up and production, as well as adherence to the most stringent quality systems, Genzyme Pharmaceuticals is a reliable partner for the supply of these additional products and services:

- Amino acid derivatives for peptides and peptidomimetics
- Synthetic peptides
- Synthetic phospholipids and related products
- Drug delivery technologies
- Custom development and GMP manufacture



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