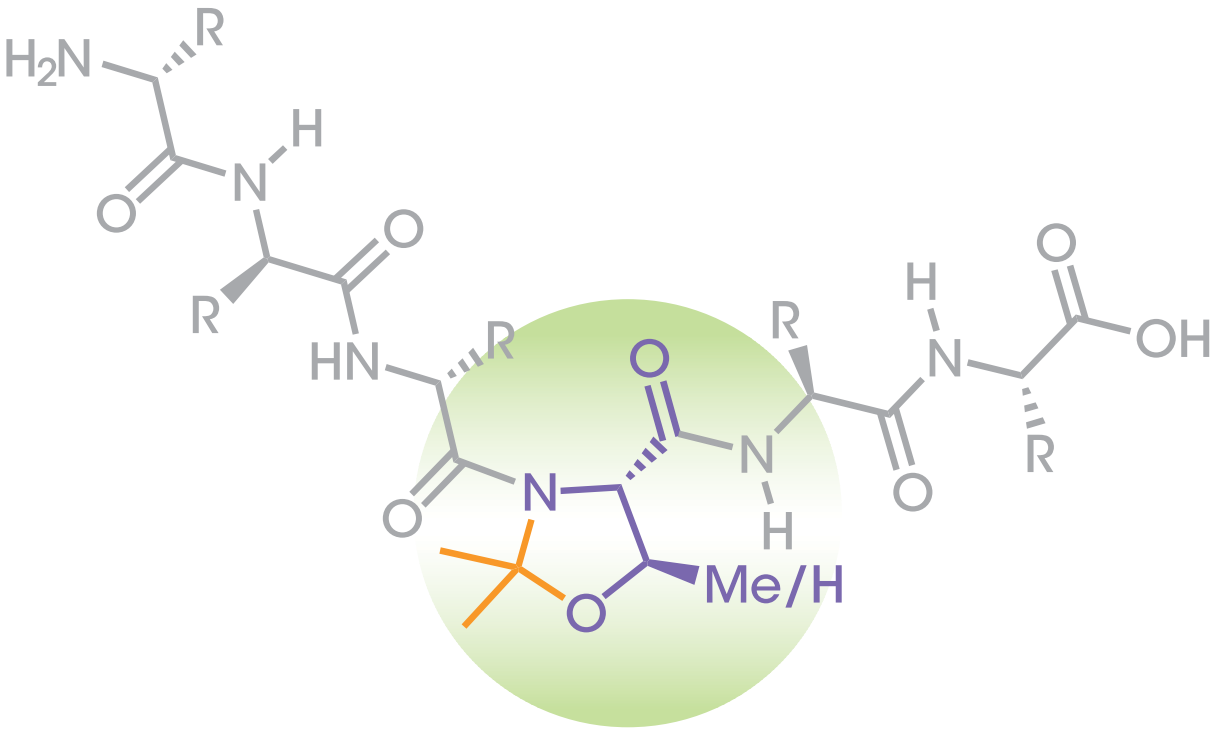
# Genzyme Pharmaceuticals

## PSEUDOPROLINE DIPEPTIDES

FOR GMP PEPTIDE PRODUCTION AND OPTIMIZED RESULTS

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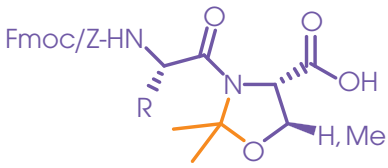
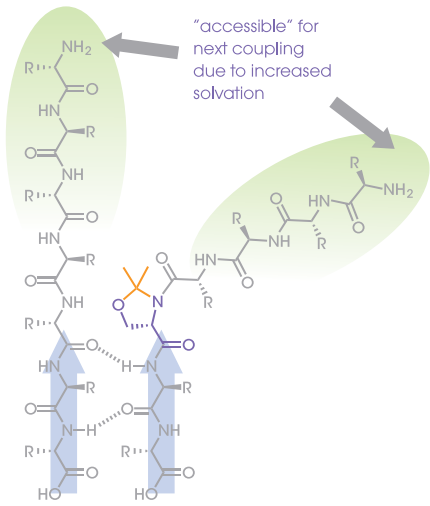
# GENZYME PHARMACEUTICALS NOW OFFERS PSEUDOPROLINES IN QUANTITIES RANGING FROM 10g TO HUNDREDS OF KILOGRAMS




Fmoc/Z-Xxx-Ser($\psi^{Me, Me}$pro)-OH
Fmoc/Z-Xxx-Thr($\psi^{Me, Me}$pro)-OH




*The presence of the proline-like ring system (fixed Φ-angle), and the preference for a cis-amide bond with the preceding residue[4], exerts a pronounced effect upon the peptide backbone, thus preventing peptide aggregation, self-association or β–structure formation.*

Genzyme Pharmaceuticals supplies amino acid derivatives of consistently high quality at attractive 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.

In light of the strong trend towards the development of peptide drugs with longer sequences and more complex structures there is a need for new technologies and building blocks to further facilitate the manufacturing process and lower overall production costs.

Despite recent improvements in large-scale production technology, the manufacture of synthetic peptides is nonetheless costly, especially for peptides with 30 or more amino acids. Low solvation during solid phase peptide synthesis (SPPS) leads to peptide chain aggregation, typically accompanied by the formation of β-sheet structures.[1] 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 Pseudoprolines (ΨPro)

*Mutter's* serine and threonine derived pseudo-proline dipeptides[2] were originally developed for bypassing the synthesis challenges associated with "difficult sequences" and long peptides[3] such as low solvation during solid phase synthesis, and limited solubility of (un)protected peptide segments. This remarkable solubilizing effect has been widely reported and referenced in scientific literature. Today, a large number of pseudoproline building blocks are commercially available and their increasing popularity as value-added protecting groups is based on evident advantages: the insertion of ΨPro building blocks saves a coupling step and improves the SPPS process (as demonstrated by higher purity of the crude peptide product) which in turn facilitates and streamlines the HPLC purification protocol.

## 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.


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

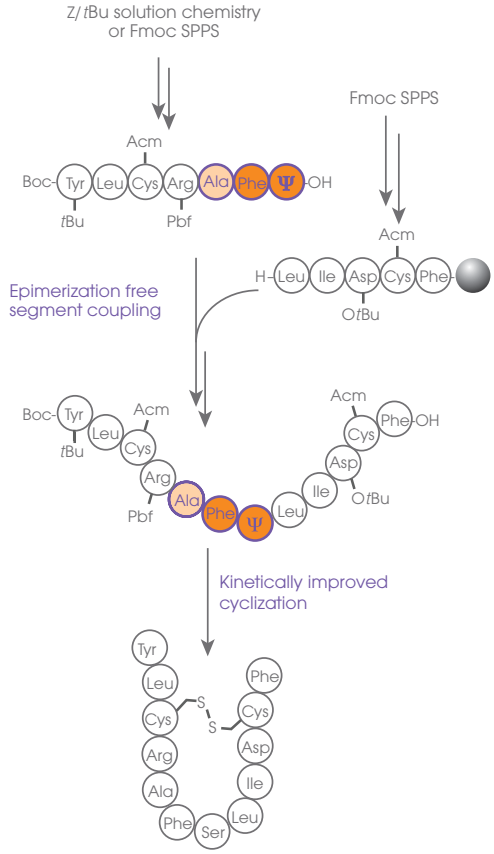
2 a) M. Mutter, A. Nefzi, T. Sato, F. Wahl, T. Wöhr, *Peptide Res.*, (1995) 8, 145-153; b) T. Wöhr, B. Rohwedder, F. Wahl, A. Nefzi, T. Sato, X. Sun, M. Mutter, *J. Am. Chem. Soc.*, (1996) 118, 9218-9227.

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 pseudoprolines offer additional segmentation sites for convergent solution /solid phase synthesis strategies. C-terminal pseudoprolines also guarantee epimerization-free segment condensation as their ring systems protect Ser and Thr from stereomutation *via* the oxazalone route. The use of ΨPro tripeptides is recommended to initiate chain elongation on solid support in order to avoid diketopiperazine formation and early cleavage from the resin at the dipeptide synthesis stage. Genzyme Pharmaceuticals' technical representatives are available to discuss the custom manufacturing of tripeptide building blocks.



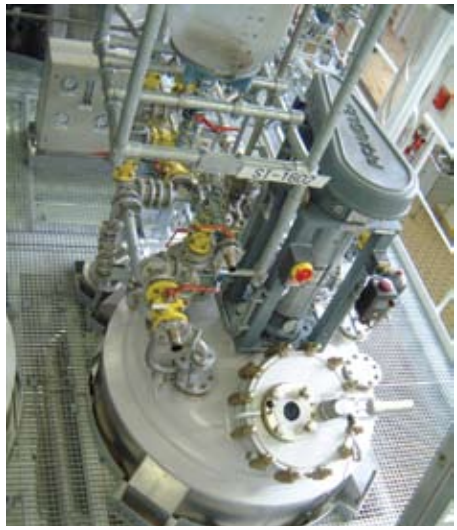

## Cyclic Peptides

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 GMP Peptide Production

Genzyme Pharmaceuticals is a pioneer in making these high-value building blocks available in consistent product quality suitable for the production of pharmaceutical-grade peptides. Pseudoproline dipeptides have important advantages for large scale peptide production:

- Greatly improved chain elongation yields, particularly for "difficult" and long sequences.
- Higher purity of crude peptide, thereby simplifying preparative HPLC purification.
- Improved overall synthesis yields.



### GENZYME PHARMACEUTICALS NOW PROVIDES A WIDE RANGE OF PSEUDOPROLINE DIPEPTIDES

Until now, limited availability of economically priced pseudoproline dipeptides in bulk quantities with consistently high purity has hampered their use as a standard protecting group for serine and threonine. Genzyme Pharmaceuticals and Neuland Laboratories have joined forces to develop economical manufacturing processes for the multi-kilogram production of tightly quality-controlled pseudoproline dipeptides. We believe that our high quality standards, together with very competitive pricing, make these the best pseudoproline building blocks currently available. Our pseudoprolines are available off-the-shelf in 10g, 25g and 100g small pack quantities. Bulk quantities are produced and supplied upon request.

## PSEUDOPROLINES AVAILABLE FROM STOCK

Genzyme Pharmaceuticals holds inventory of 28 different pseudoproline building blocks that can be ordered in units of 10g, 25g and 100g. The products ship with analytical result sheets certifying chemical purity, enantiomeric purity, actual dipeptide content, water content and other important quality parameters of each production batch. Please contact Genzyme Pharmaceuticals for information on pricing.

| PRODUCT DESCRIPTION | PRODUCT CODE |
|---|---|
| **Serine Pseudoprolines** | |
| Fmoc-Ala-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-182 |
| Fmoc-Asn(Trt)-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-232 |
| Fmoc-Asp(O*t*Bu)-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-215 |
| Fmoc-Gln(Trt)-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-226 |
| Fmoc-Glu(O*t*Bu)-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-227 |
| Fmoc-Gly-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-183 |
| Fmoc-Ile-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-207 |
| Fmoc-Leu-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-166 |
| Fmoc-Lys(Boc)-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-228 |
| Fmoc-Phe-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-185 |
| Fmoc-Ser(*t*Bu)-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-155 |
| Fmoc-Trp(Boc)-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-229 |
| Fmoc-Tyr(*t*Bu)-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-195 |
| Fmoc-Val-Ser($\Psi^{Me,Me}$pro)-OH | DP-05-184 |
| **Threonine Pseudoprolines** | |
| Fmoc-Ala-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-169 |
| Fmoc-Asn(Trt)-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-233 |
| Fmoc-Asp(O*t*Bu)-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-162 |
| Fmoc-Gln(Trt)-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-206 |
| Fmoc-Glu(O*t*Bu)-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-219 |
| Fmoc-Gly-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-168 |
| Fmoc-Ile-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-163 |
| Fmoc-Leu-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-167 |
| Fmoc-Lys(Boc)-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-221 |
| Fmoc-Phe-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-158 |
| Fmoc-Ser(*t*Bu)-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-181 |
| Fmoc-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-230 |
| Fmoc-Tyr(*t*Bu)-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-231 |
| Fmoc-Val-Thr($\Psi^{Me,Me}$pro)-OH | DP-05-170 |

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