TESAMORELIN — INSERT A SINGLE I,I+4 HYDROCARBON STAPLE BETWEEN POSITIONS 13 (VAL→S5) AND 17 (LEU→S5) ON THE CENTRAL A-HELIX, REPLACING VAL-13 AND LEU-17 WITH (S)-2-(4'-PENTENYL)ALANINE RESIDUES CROSS-LINKED VIA RUTHENIUM-CATALYZED RING-CLOSING METATHESIS TO FORM AN ALL-HYDROCARBON BRIDGE ACROSS THE SOLVENT-EXPOSED FACE OF THE HELIX

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DISCARDED PERFORMANCE

INSERT A SINGLE I,I+4 HYDROCARBON STAPLE BETWEEN POSITIONS 13 (VAL→S5) AND 17 (LEU→S5) ON THE CENTRAL A-HELIX, REPLACING VAL-13 AND LEU-17 WITH (S)-2-(4'-PENTENYL)ALANINE RESIDUES CROSS-LINKED VIA RUTHENIUM-CATALYZED RING-CLOSING METATHESIS TO FORM AN ALL-HYDROCARBON BRIDGE ACROSS THE SOLVENT-EXPOSED FACE OF THE HELIX

GROWTH HORMONE-RELEASING HORMONE RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
45.8%	0.368 / 0.171	DISCARDED

TARGET	UNIPROT	BINDING PROBABILITY
Growth hormone-releasing hormone receptor	Q02643	—

TLDR

Fold №50 applies an i,i+4 all-hydrocarbon staple between positions 13 and 17 of Tesamorelin's central α-helix, aiming to pre-organize the bioactive helical conformation for superior GHRHR engagement. AlphaFold2 returned a global pLDDT of 0.46 and an ipTM of 0.17, both well below thresholds for interpretable structural inference, rendering the docking pose uninformative. This continues a pattern established in Folds №13 and №29, where Tesamorelin helix-rigidification strategies via Aib substitution also failed to produce confident predictions. The prediction infrastructure is not yet equipped to handle the non-canonical (S)-2-(4'-pentenyl)alanine staple residues, meaning this is a tool limitation rather than a chemical verdict on the staple concept itself.

EXECUTIVE SUMMARY

Tesamorelin helix staple (Val-13/Leu-17 → S5 i,i+4): pLDDT 0.46, ipTM 0.17 — uninformative. The predictor cannot handle non-canonical S5 cross-link residues. The chemical concept is mechanistically sound; wet-lab synthesis and CD/protease validation are the appropriate next step.

DETAILED ANALYSIS

Tesamorelin is a 44-amino acid synthetic analogue of human growth hormone-releasing hormone (GHRH), distinguished by an N-terminal trans-3-hexenoyl pharmacophore that confers partial DPP-IV resistance while preserving agonist activity at the GHRHR (UniProt Q02643). Approved by the FDA in 2010 for HIV-associated lipodystrophy, its primary clinical liabilities are proteolytic lability requiring daily subcutaneous injection and conformational flexibility in the free-peptide state that may reduce binding efficiency relative to a pre-organized helical scaffold. The current fold addresses these liabilities by attempting a covalent conformational lock via hydrocarbon stapling — a well-validated strategy in the peptide drug discovery field most prominently exemplified by ALRN-6924, a stapled p53 activator that reached clinical trials.

The modification rationale is mechanistically sound in principle. Positions 13 (Val) and 17 (Leu) were selected because they sit on the same hydrophobic face of the

predicted amphipathic central helix, putatively away from the GHRHR-contacting residues, making them plausible staple attachment points that should not sterically occlude receptor engagement. The i,i+4 spacing is the classical all-hydrocarbon staple geometry, and (S)-2-(4'-pentenyl)alanine (S5) residues cross-linked via ruthenium-catalyzed ring-closing metathesis have been used across dozens of stapled peptide programmes. The hypothesis is well-constructed and represents a genuine conceptual advance over the Aib substitution approaches tested in Folds №13 and №29, which nucleate helicity locally but do not covalently enforce it.

Unfortunately, the structure prediction infrastructure encountered the same barrier that sank those earlier Tesamorelin folds: the global pLDDT of 0.46 is nearly identical to Fold №13 (0.49) and Fold №29 (0.47), and the ipTM of 0.17 is far below the ≥0.50 threshold conventionally required for any confidence in a modelled protein–protein interface. These metrics indicate that AlphaFold2 is not producing a stable, self-consistent fold for the stapled peptide in complex with GHRHR, and the resulting coordinates cannot be interpreted as evidence for or against helical pre-organization. The hypothesis specifically predicted overall pLDDT ≥ 0.65 with the helix segment exceeding 0.75; neither target was approached.

The most likely technical explanation is that the non-canonical (S)-2-(4'-pentenyl)alanine residues are outside AlphaFold2's training distribution. The model has essentially no learned representations for unnatural amino acids with extended aliphatic sidechains and covalent cross-links; it likely interprets the S5 residues as noise or defaults to a disordered prediction rather than extrapolating the helical geometry that the staple would physically enforce in a real molecule. This is a fundamental limitation of sequence-based neural network predictors for chemically modified peptides — a limitation that also manifested in Fold №42 (Sermorelin lactam staple, pLDDT 0.50, DISCARDED) for similar reasons. The pattern across Folds №13, №29, №42, and now №50 consistently shows that conformational-lock strategies on GHRHR-targeting peptides produce low-confidence predictions, likely because the model cannot accommodate the non-canonical chemistry driving the modification's value.

The literature context does not undermine the hypothesis — it simply provides no direct data to evaluate it. No published structural data on the tesamorelin–GHRHR complex appears in the retrieved corpus, and no SAR studies for the central helix of GRF(1-29) exist in these papers. The clinical motivation (protease resistance, daily injection burden) is robustly supported, and the broader stapling literature (outside this retrieved set) provides strong precedent for the approach. The heuristic stability score of 0.378 and long half-life estimate are consistent with a moderately stable peptide, but these are sequence-based estimates that do not account for the actual staple chemistry.

This fold should be read as a tool failure, not a chemical failure. The staple concept is arguably the most mechanistically sophisticated Tesamorelin modification yet attempted in this lab, and the prediction system simply cannot evaluate it. What

would be needed to generate meaningful signal is either a physics-based modelling approach (molecular dynamics with explicit staple force-field parameters), a dedicated non-canonical amino acid predictor, or wet-lab synthesis and biophysical characterisation. The negative prediction is not informative about the molecule's actual helicity, receptor affinity, or proteolytic stability.

In the broader context of the lab's running narrative, Fold №50 completes a trio of failed Tesamorelin helix-rigidification predictions (Folds №13, №29, №50) alongside a failed Sermorelin stapling attempt (Fold №42). Together these establish a clear empirical pattern: current in silico tools are not equipped to evaluate conformational-lock strategies on GHRHR-targeting long-chain peptides. Future Tesamorelin exploration should either pivot to modification chemistries within the model's training distribution (e.g., conservative natural amino acid substitutions, PEGylation endpoints) or accept that the staple hypothesis requires wet-lab validation rather than in silico triage.

RESEARCH BRIEF

FOLD №50 — TESAMORELIN HEXA-POSITION HYDROCARBON STAPLE (VAL-13/LEU-17 → S5 I,I+4)

Verdict: DISCARDED | pLDDT 0.46 | ipTM 0.17

MECHANISM OF ACTION (BACKGROUND)

Tesamorelin is a 44-amino acid synthetic analogue of human growth hormone-releasing hormone (GHRH), carrying an N-terminal trans-3-hexenoyl pharmacophore that protects against DPP-IV cleavage at Ala-2. It acts as a full agonist at the GHRHR (UniProt Q02643), stimulating pulsatile GH secretion from anterior pituitary somatotrophs. The GH pulse drives downstream IGF-1 production, lipolysis in visceral adipose tissue, and the hepatic/metabolic endpoints that make Tesamorelin clinically valuable in HIV-associated lipodystrophy.

The peptide's central segment (residues ~9–22 of the GRF(1-29) core) is predicted to adopt an amphipathic α-helix that docks into the GHRHR extracellular domain. In solution, however, the free peptide samples substantially disordered states, potentially reducing binding affinity relative to the bound-state conformation. The trans-3-hexenoyl cap provides N-terminal stability but does nothing to pre-organize the central helix — this is the conformational vulnerability the current fold aimed to address.

MODIFICATION HYPOTHESIS (WHAT WE TESTED)

This fold installed a single i,i+4 all-hydrocarbon staple between positions 13 (Val → (S)-2-(4'-pentenyl)alanine, S5) and 17 (Leu → S5), cross-linked via ruthenium-catalyzed ring-closing metathesis to form a covalent macrocyclic bridge across the putative solvent-exposed face of the central helix. The hypothesis was that this covalent lock would:

1. **Pre-organize the bioactive α-helical conformation**, reducing conformational entropy upon GHRHR binding and potentially increasing binding affinity
2. **Confer protease resistance** along the central helix, complementing the N-terminal trans-3-hexenoyl cap
3. **Preserve the N-terminal pharmacophore and C-terminal recognition motif**, since the staple was placed on a solvent-exposed hydrophobic face away from predicted receptor contact residues

The approach was deliberately designed to avoid the Aib substitution chemistry that failed in **Fold №13** (Gln-8 → Aib, pLDDT 0.49, DISCARDED) and **Fold №29** (Ala-2 → Aib, pLDDT 0.47, DISCARDED), and to address a different failure mode — those modifications nucleate helicity locally but cannot covalently enforce it across the full helix span.

WHY THE PREDICTION WAS UNINFORMATIVE (TECHNICAL ANALYSIS)

Metric	Predicted	Target	Verdict
Global pLDDT	0.46	≥ 0.65	✗ Far below threshold
Helix-region pLDDT (9–22)	~0.46 (no improvement)	≥ 0.75	✗ Not observed
ipTM (interface confidence)	0.17	≥ 0.50	✗ No interpretable interface
pTM (global fold)	0.37	—	Marginal

The core technical problem is that (S)-2-(4'-pentenyl)alanine (S5) residues are entirely outside AlphaFold2's training distribution. The model has no learned representations for unnatural amino acids with extended aliphatic side-chains and covalent ring-closing cross-links. Presented with two S5 positions and an implicit covalent bridge, the predictor almost certainly interprets these residues as disordered noise, defaulting to a low-confidence unstructured prediction rather than extrapolating the helical geometry that the physical staple would enforce.

This is the same barrier that produced the DISCARDED verdict in **Fold №42** (Sermorelin Lys-21/Asp-25 i,i+4 lactam staple, pLDDT 0.50, DISCARDED). That fold involved a different staple chemistry (lactam vs. hydrocarbon) and a different peptide, but the structural predictor responded identically — with a low-confidence, interface-uninformative output. Together, Folds №42 and №50 establish a reproducible empirical finding: **current AF2-based tools cannot evaluate conformational-lock strategies on GHRHR-targeting peptides involving non-canonical cross-linking chemistry.**

The heuristic sequence-based profile (aggregation propensity 0.141, stability score 0.378, long half-life estimate) provides no useful signal here because it does not account for the actual staple geometry, cross-link rigidity, or the protease-shielding effect of the hydrocarbon bridge. These numbers describe the underlying Tesamorelin sequence, not the stapled analogue.

WHAT THIS TELLS US (NEGATIVE RESULTS ARE DATA)

This fold rules out one thing confidently: **in silico triage of hydrocarbon-stapled Tesamorelin analogues is not currently feasible with this prediction stack.** This is a meaningful finding for lab workflow — it means any future stapled GHRH-family peptide must be routed directly to wet-lab validation rather than filtered computationally.

It does not tell us that the staple concept is chemically flawed. The scientific rationale — covalent helix pre-organization at positions on the solvent-exposed face — remains mechanistically plausible and draws on a well-validated general framework (Verdine/Walensky chemistry, clinical precedent in ALRN-6924). The absence of an interpretable in silico signal is not evidence of failure in the molecule; it is evidence of a tool capability gap.

The broader pattern across Tesamorelin folds (№13, №29, №50, all DISCARDED) and the related Sermorelin staple (Fold №42, DISCARDED) establishes a lab-level insight: **GHRHR-targeting long-chain peptides with non-canonical backbone modifications consistently defeat current structure predictors.** Folds targeting smaller peptides with more conventional modifications — such as Ipamorelin with palmitoyl lipidation (Fold №48, REFINED, pLDDT 0.78) or Ipamorelin macrocyclization (Fold №33, REFINED, pLDDT 0.73) — do produce meaningful signal. The predictor succeeds on pentapeptide GHSR-1a ligands with compatible chemistry; it fails on 44-mer GHRHR ligands with non-canonical cross-links.

ALTERNATIVE HYPOTHESES TO TEST (AVOIDING THE FAILURE MODE)

Route A — Wet-lab bypass (recommended for staple concept): Synthesize the Val-13(S5)/Leu-17(S5) stapled Tesamorelin analogue directly. Measure helicity by CD spectroscopy, protease resistance by HPLC-based degradation assay (DPP-IV, chymotrypsin, plasma stability), and GHRHR binding by a competitive radioligand displacement assay. The hypothesis is strong enough on mechanistic grounds to warrant this without in silico validation.

Route B — Physics-based modelling: Apply molecular dynamics simulation with explicit CHARMM or AMBER force-field parameters for S5 residues and the olefinic cross-link. This would directly test the helicity pre-organization hypothesis in silico without relying on AF2's sequence-based prediction architecture.

Route C — Predictor-compatible Tesamorelin modifications: Pivot to modification chemistries within AF2's training distribution. Options that have not been tested on Tesamorelin in this lab include: C-terminal PEGylation for half-life extension (does not involve non-canonical backbone chemistry); conservative substitution of Ser-9 or Asn-8 with natural proteolysis-resistant residues; or fatty acid conjugation at an internal Lys residue analogous to the successful palmitoylation in Fold №48 (Ipamorelin, REFINED). These would generate interpretable predictions that could guide synthetic prioritization.

Route D — Shorter GRF fragment stapling: Test the i,i+4 hydrocarbon staple concept on a truncated GRF(1-17) fragment rather than the full 44-mer. Shorter stapled peptides are better represented in AF2's training data and may yield interpretable predictions, with the understanding that C-terminal truncation may affect GHRHR efficacy.

SEQUENCES

NATIVE

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YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL
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MODIFIED

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Y(hexenoyl)-ADAIFTNSYRK-X(S5)-LGQ-X(S5)-SARKLLQDIMSRQQGESNQERGARARL [staple
between pos 13 and 17]
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled)
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- (S)-2-(4'-pentenyl)alanine (S5) staple residues are outside AlphaFold2's training distribution — the low pLDDT almost certainly reflects model incompatibility with non-canonical cross-linking chemistry, not chemical infeasibility of the staple
- ipTM 0.17 means no interpretable peptide–receptor interface was predicted; no conclusions about binding pose, staple face orientation, or receptor contact preservation can be drawn
- heuristic stability score, aggregation propensity, and half-life estimates are sequence-based and do not account for the conformational or protease-shielding effects of the hydrocarbon staple cross-link
- no published cryo-EM or co-crystal structure of tesamorelin–GHRHR complex is available; the assumption that Val-13 and Leu-17 are on the solvent-exposed non-contacting face remains unverified in the literature retrieved

CITATIONS

1. **PMID —** (2024) — — Efficacy and safety of tesamorelin in people with HIV on integrase inhibitors.
2. **PMID —** (2011) — — Tesamorelin.
3. **PMID —** (2012) — — Tesamorelin: a growth hormone-releasing factor analogue for HIV-associated lipodystrophy.
4. **PMID —** (2011) — — Tesamorelin: a review of its use in the management of HIV-associated lipodystrophy.
5. **PMID —** (2009) — — Tesamorelin, a human growth hormone releasing factor analogue.
6. **PMID —** (2026) — — Safety and Efficacy of Approved and Unapproved Peptide Therapies for Musculoskeletal Injuries and Athletic Performance
7. **PMID —** (2026) — — Evaluation of Research Grade Peptides Marketed Directly to Consumers Reveals Extensive Variability in Purity and Measured Abundance

SOLANA SIGNATURE 2iU52mPods1MYdtbu9CkFip12rwV8YYG6Va7UJ9WXNxiKJ5JdEW3W2mPXp1wLijZrHSHjvu1RB1tJn33xjkxhk2z

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