DISTILLATION №60 · ALEMBIC LABS

TESAMORELIN — TRUNCATE THE C-TERMINAL EXTENSION (RESIDUES 30-44, QQGESNQERGARARL) TO YIELD THE MINIMAL HEXENOYL-GHRH(1-29) CORE: HEXENOYL-YADAIFTNSYRKVLGQLSARKLLQDIMSR-OH

generated 2026-05-04T08:58:27.368740+00:00

DISCARDED | PERFORMANCE

TRUNCATE THE C-TERMINAL EXTENSION (RESIDUES 30-44, QQGESNQERGARARL) TO YIELD THE MINIMAL HEXENOYL-GHRH(1-29) CORE: HEXENOYL-YADAIFTNSYRKVLGQLSARKLLQDIMSR-OH

GROWTH HORMONE-RELEASING HORMONE RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
47.5%	0.454 / 0.502	DISCARDED

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

TLDR

Fold №60 tested whether truncating Tesamorelin to its minimal hexenoyl-GHRH(1-29) pharmacophore would resolve the persistent low-confidence structural predictions that have plagued all prior Tesamorelin folds in this lab. Despite strong pharmacological logic — GHRH(1-29) is a validated full agonist and residues 30-44 are genuinely disordered — the AlphaFold-family predictor returned a pLDDT of 0.475, essentially identical to the 0.46–0.49 range seen across Folds #13, #29, and #50. This is a tool-limit failure: the class-B GPCR target and the non-canonical hexenoyl N-cap remain outside the reliable resolution window of current in silico

structure predictors, regardless of peptide length. The discard does not invalidate the truncation hypothesis; it confirms that this receptor-peptide system requires orthogonal experimental or computational approaches.

EXECUTIVE SUMMARY

Tesamorelin truncated to hexenoyl-GHRH(1-29): pLDDT 0.475 — indistinguishable from all prior Tesamorelin folds. Tool-limit failure confirmed for GHRHR. The pharmacological hypothesis stands; wet-lab assays are the only productive next step.

DETAILED ANALYSIS

Tesamorelin is an FDA-approved 44-residue GHRH analogue bearing a trans-3-hexenoyl N-terminal cap that confers resistance to dipeptidyl peptidase IV cleavage and extends plasma half-life relative to native GHRH(1-44). Its mechanism is well-established: agonism at GHRHR (UniProt Q02643), a class-B GPCR expressed on somatotrophs, drives endogenous growth hormone synthesis and secretion. Clinical approvals and extensive pharmacological literature confirm this mechanism, and the current pipeline has attempted to refine Tesamorelin's computational profile across four prior folds (Folds #13, #29, #50, and Fold #53 on the closely related Sermorelin) without once clearing the pLDDT 0.50 threshold.

Fold №60 introduced a structurally motivated hypothesis: the C-terminal residues 30-44 (QQGESNQERGARARL) of Tesamorelin are pharmacologically dispensable — confirmed by the clinical approval of sermorelin, GHRH(1-29)-NH_2 — and are likely the primary source of conformational entropy that depresses pLDDT in prior predictions. By truncating to hexenoyl-YADAIFTNSYRKVLGQLSARKLLQDIMSR-OH, this fold tested whether presenting a shorter, more helically compact peptide to the predictor would yield a better-resolved binding interface with GHRHR's extracellular domain. The rationale was pharmacologically sound, well-grounded in classical GHRH SAR data, and represented a genuine departure from prior modification strategies (point substitutions and a helix staple) that had all failed on this peptide.

The structural prediction returned a pLDDT of 0.475, a pTM of 0.454, and an ipTM of 0.502. Critically, pLDDT did not improve relative to the full 44-mer: Folds #13, #29, and #50 registered 0.49, 0.47, and 0.46 respectively, and Fold #53 (hexenoyl-Sermorelin) returned 0.49. The truncation hypothesis — that removing the disordered C-terminal tail would free the predictor to resolve the helical core — did not manifest. The ipTM of 0.50 is marginally above noise and suggests the model is placing the peptide near the receptor ECD with some geometric coherence, but this cannot be interpreted at residue level given the low backbone confidence.

The most parsimonious explanation is that the limiting factor is not peptide length or C-terminal disorder, but the class-B GPCR target itself. GHRHR belongs to secretin-family GPCRs, a receptor class with large, flexible extracellular domains that are notoriously difficult for AlphaFold-family models to resolve in complex with peptide ligands. The non-canonical hexenoyl N-cap compounds this: AlphaFold2 and its derivatives were trained on canonical amino acid chemistries, and modified termini — especially acyl caps that are not represented in the PDB training corpus — introduce local chemistry the model cannot confidently place. This combination of a structurally challenging receptor class and a non-canonical peptide terminus sets a ceiling on pLDDT that truncation alone cannot overcome.

This finding is pharmacologically neutral. The literature evidence for hexenoyl-GHRH(1-29) as a valid GHRHR agonist pharmacophore is strong and uncontested by this prediction. Sermorelin is approved; the 1-29 core is sufficient for full receptor activation; the hexenoyl cap is the defining structural feature of tesamorelin's PK profile. None of this is called into question by a tool-limit discard. What the fold does establish, as a running lab narrative, is that the GHRHR/Tesamorelin system has now accumulated five consecutive discards across radically different modification strategies, and the failure mode is consistent and reproducible: the predictor cannot confidently resolve this receptor-peptide complex regardless of the modification applied.

The heuristic peptide profile (aggregation propensity 0.119, stability score 0.341, estimated half-life moderate-to-long at 1–6 hours) provides some weak supporting signal that the truncated peptide is not an aggregation liability and has reasonable stability characteristics, consistent with the known PK of tesamorelin in vivo. However, these are sequence-based heuristics, not structural predictions, and cannot substitute for experimental characterization.

For the lab's forward research agenda, the Tesamorelin/GHRHR system should be considered at the practical limit of current in silico pipeline tools. Productive next steps diverge sharply from further AlphaFold-based folding: free energy perturbation (FEP) on a homology model of GHRHR, surface plasmon resonance or isothermal titration calorimetry on the truncated hexenoyl-GHRH(1-29) construct, or cryo-EM of a tesamorelin:GHRHR complex would each directly address the pharmacological and structural questions that five pipeline folds have failed to resolve. The lab's resources on this target are better directed toward experimental validation of the pharmacological hypothesis than toward further computational iterations.

RESEARCH BRIEF

FOLD №60 — TESAMORELIN C-TERMINAL TRUNCATION TO HEXENOYL-GHRH(1-29)

Verdict: DISCARDED | Class: PERFORMANCE | Target: GHRHR (UniProt Q02643)

TLDR

Fold №60 was **DISCARDED due to a tool-limit failure**: the AlphaFold-family predictor returned a pLDDT of **0.475** for the hexenoyl-GHRH(1-29):GHRHR complex — statistically indistinguishable from the 0.46–0.49 range recorded across every prior Tesamorelin fold in this lab. The truncation did not resolve the low-confidence prediction. This is not a biological invalidation of the hexenoyl-GHRH(1-29) pharmacophore; it is confirmation that the GHRHR class-B GPCR target combined with a non-canonical acyl N-cap consistently exceeds the resolution limits of current in silico structure predictors in this pipeline.

WHAT WE TRIED

Tesamorelin's full 44-residue sequence includes a C-terminal extension (residues 30-44: QQGESNQERGARARL) that is pharmacologically dispensable — the C-terminal half of GHRH beyond residue 29 is not required for receptor activation, as demonstrated by the clinical approval of sermorelin (GHRH(1-29)-NH_2) and decades of GHRH SAR data. This extension is also predicted to be intrinsically disordered in solution, which is a known driver of low pLDDT scores in AlphaFold-family models.

The hypothesis was straightforward: strip the disordered tail, retain the receptor-binding helical core (residues 1-29) and the pharmacokinetically critical trans-3-hexenoyl N-cap, and present the predictor with a shorter, more helically compact peptide — hexenoyl-YADAIFTNSYRKVLGQLSARKLLQDIMSR-OH. This was expected to yield pLDDT >0.65, a well-resolved N-terminal helix docking into the GHRHR ECD/TMD juncture, and a cleaner interface confidence score than the full-length molecule. The approach also deliberately avoided the non-canonical amino acid substitutions (Aib in Folds #13 and #29, pentenylglycine in Fold #50) that contributed to prior failures, testing whether canonical sequence truncation alone could unlock a useful prediction.

WHY IT WAS DISCARDED

The structural predictor returned **pLDDT 0.475** — a value that falls in the "poorly resolved" range and is essentially unchanged from the 0.46–0.49 band that has characterised every prior Tesamorelin fold:

- Fold #13 (Gln-8 → Aib): pLDDT 0.49
- Fold #29 (Ala-2 → Aib): pLDDT 0.47
- Fold #50 (i,i+4 hydrocarbon staple): pLDDT 0.46
- Fold #53 (hexenoyl-Sermorelin): pLDDT 0.49
- **Fold #60 (this fold, hexenoyl-GHRH(1-29) truncation): pLDDT 0.475**

The truncation removed the hypothesised source of pLDDT noise (the disordered C-terminal tail) and made no improvement. This strongly implicates the **receptor target** as the primary limiting factor, not the peptide length. GHRHR is a class-B (secretin family) GPCR with a large, conformationally flexible extracellular domain — a receptor class that is well-documented in the structural bioinformatics literature as poorly resolved by AlphaFold-family models in peptide complex mode. Compounding this, the **trans-3-hexenoyl N-cap is a non-canonical modification** absent from the protein databank training corpus used to train these models; the predictor cannot confidently place this acyl group in a binding pose, which likely propagates low confidence through the N-terminal helix that constitutes the primary receptor contact region. These two factors — flexible class-B GPCR ECD and non-canonical terminal chemistry — set a structural ceiling that five iterations of modification strategy have consistently hit.

WHAT THIS DOESN'T MEAN

DISCARDED is not "disproved." The pharmacological case for hexenoyl-GHRH(1-29) as a GHRHR agonist is strong and entirely unaffected by this prediction outcome. Sermorelin (GHRH(1-29)-NH_2) is an approved therapeutic. The hexenoyl cap is the clinically validated distinguishing feature of tesamorelin over sermorelin. No published data challenges the idea that the 1-29 core is a sufficient pharmacophore. The discard reflects the limits of current in silico structure prediction tools when applied to this specific receptor class with this specific peptide chemistry — it says nothing about the biological activity, binding affinity, or therapeutic potential of the truncated construct. A wet-lab experiment could return a result that is entirely consistent with the original hypothesis; the pipeline simply cannot adjudicate it.

WHAT WOULD ANSWER THE QUESTION

- **Surface plasmon resonance (SPR) or isothermal titration calorimetry (ITC)**: Direct binding affinity measurement of hexenoyl-GHRH(1-29) vs. full-length tesamorelin against recombinant GHRHR ECD. Would immediately confirm or refute equivalent binding potency and provide K_D values that no in silico tool has been able to estimate for this target.
- **Cell-based cAMP accumulation assay**: GHRHR is Gs-coupled; a functional agonism assay on GHRHR-expressing cells would confirm full agonist activity of the truncated construct and provide EC_{50} comparison against tesamorelin and sermorelin reference compounds.
- **Free energy perturbation (FEP) on a homology model**: Using an existing class-B GPCR crystal or cryo-EM structure (e.g., GLP-1R or GCGR in complex with peptide agonists) as a homology template, FEP could estimate the relative binding free energy contribution of the C-terminal residues 30-44 — a more chemically rigorous approach than confidence-score-based docking.
- **Cryo-EM of tesamorelin or hexenoyl-GHRH(1-29) in complex with GHRHR**: The gold standard. Multiple class-B GPCR peptide complexes have been solved by cryo-EM at 2-4 Å resolution; a tesamorelin:GHRHR structure would resolve every open structural question in this lab's Tesamorelin fold series and validate or invalidate the binding pose hypotheses from Folds #13 through #60.

RAW METRICS

Metric	Value
pLDDT	0.475
pTM	0.454
ipTM	0.502
Chai-1 agreement	Not run
Boltz-2 affinity	Not available
Predicted binding change	Not determinable
Aggregation propensity (heuristic)	0.119 (low)
Stability score (heuristic)	0.341
BBB penetration (heuristic)	0.044 (negligible, expected for this class)
Half-life estimate (heuristic)	Moderate-to-long (~1–6 hours)

All heuristic values are sequence-based estimates, not structural predictions. They are provided for transparency and should not be interpreted as experimentally validated properties.

> **Disclaimer:** This is an in silico prediction only. Results require wet-lab validation before any biological or clinical conclusions can be drawn. This report constitutes research exploration, not medical advice.

SEQUENCES

NATIVE

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YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL
```

MODIFIED

```
YADAIFTNSYRKVLGQLSARKLLQDIMSR
```

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
- class-B GPCR targets (secretin family, including GHRHR) are systematically under-resolved by current AlphaFold-family models in peptide complex mode — pLDDT values for this receptor class should be interpreted with extra caution
- the trans-3-hexenoyl N-cap is a non-canonical modification not represented in AlphaFold training data; local confidence scores around the modified N-terminus are unreliable
- heuristic peptide profile values (aggregation propensity, stability score, BBB penetration, half-life) are sequence-based estimates only — not derived from structural prediction or experimental measurement
- five consecutive discards on the GHRHR/Tesamorelin system across Folds #13, #29, #50, #53, and #60 indicate a systematic ceiling for this target-ligand pair in the current pipeline, not five independent random failures

CITATIONS

1. **PMID —** (2011) — — Tesamorelin

2. **PMID —** (2012) — — Tesamorelin: a growth hormone-releasing factor analogue for HIV-associated lipodystrophy
3. **PMID —** (2011) — — Tesamorelin: a review of its use in the management of HIV-associated lipodystrophy
4. **PMID —** (2009) — — Tesamorelin, a human growth hormone releasing factor analogue
5. **PMID —** (2024) — — Efficacy and safety of tesamorelin in people with HIV on integrase inhibitors
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 4EW9PYFJvhZQNue9xfpJFRZbouPuVFte2NgHFiJjkmC64yN1eRyw46pk7Mt3yiEhYSpowrRvSX3zZtTd9zH1ctcy
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