DISTILLATION №13 · ALEMBIC LABS

TESAMORELIN — GLN-8 → AIB (2-AMINOISOBUTYRIC ACID) SUBSTITUTION

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DISCARDED | PERFORMANCE | GLN-8 → AIB (2-AMINOISOBUTYRIC ACID) SUBSTITUTION | GROWTH HORMONE-RELEASING HORMONE RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
49.2%	0.441 / 0.340	DISCARDED

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

TLDR

Fold №13 tests whether substituting Gln-8 with the helix-inducing non-proteogenic amino acid Aib in Tesamorelin can pre-organize the N-terminal α-helix for improved GHRHR engagement and proteolytic stability. The structure prediction returned a peptide pLDDT of 0.49 and an ipTM of 0.34, indicating low backbone confidence and an unreliably resolved docking pose — insufficient to confirm or deny the helix-reinforcement hypothesis. The predicted outcome of pLDDT >0.85 in residues 4–13 was not achieved, and no measurable binding signal was produced. The modification hypothesis remains mechanistically coherent but computationally intractable with the current toolchain.

EXECUTIVE SUMMARY

Tesamorelin Gln-8→Aib: pLDDT 0.49, ipTM 0.34 — below confidence threshold for any structural verdict. The helix-stabilization hypothesis is mechanistically coherent but computationally intractable at this peptide length and receptor class with the current toolchain.

DETAILED ANALYSIS

Tesamorelin is a 44-residue synthetic analogue of human GHRH(1-44), distinguished by its trans-3-hexenoyl N-terminal cap, which protects against DPP-IV cleavage at the Tyr-1/Ala-2 bond. It is the only FDA-approved therapy for HIV-associated lipodystrophy and operates through direct GHRHR engagement to stimulate pulsatile GH secretion and reduce visceral adipose tissue. The clinical literature consistently establishes its efficacy across multi-year trials, including on modern integrase inhibitor-based ART regimens, confirming the GHRHR pathway as robustly druggable. This fold attempts to build on tesamorelin's already-solid pharmacological foundation by addressing a second, complementary axis of optimization: conformational rigidity of the N-terminal helix.

The structural rationale for this distillation is grounded in the well-established role of the N-terminal α-helix (approximately residues 1–13) in GHRH receptor engagement. The amphipathic character of this region drives productive contacts with the GHRHR extracellular domain, and disruption of helical geometry attenuates potency. Position 8 (Gln) sits on the solvent-exposed face of this helix and is not known to make direct receptor contacts, making it a structurally logical site for a helix-stabilizing substitution. Aib's geminal dimethyl group on the α-carbon enforces φ/ψ dihedral angles within the canonical α-helical region, and its helix-inducing properties are extensively validated across peptide chemistry literature. The hypothesis is that pre-organizing residues 1–13 into the bioactive conformation would reduce entropic costs of receptor binding and potentially slow mid-helix proteolytic attack.

This modification is intentionally distinct from prior strategies explored in the lab. The Sermorelin D-Ala-2 fold (Fold №2, DISCARDED, pLDDT 0.49) targeted DPP-IV blockade via stereochemical inversion at the N-terminus — a strategy already addressed in tesamorelin by the trans-3-hexenoyl cap itself. The Ipamorelin N-Me-Aib fold (Fold №4, REFINED, pLDDT 0.80) demonstrated that Aib-class chemistry is compatible with GH-axis receptor engagement and can yield high-confidence predicted structures in a related peptide class. Fold №13 sought to apply similar conformational logic to a mid-helix position in a longer, clinically validated peptide, targeting a different stability axis entirely.

The structure prediction results, however, were not informative. The peptide pLDDT of 0.491 is virtually identical to the Sermorelin D-Ala-2 result and sits well below the threshold for confident backbone placement. The ipTM of 0.34 indicates the predicted peptide-receptor interface pose is not reliably resolved — the model does not converge on a stable docking geometry. No Chai-1 agreement data or Boltz-2 affinity values were produced, removing the secondary confidence checks that would ordinarily be used to triangulate a weak primary signal. The predicted outcome of pLDDT >0.85 across residues 4–13 was not achieved by any metric.

The heuristic sequence-based property profile offers modest contextual data but does not rescue the verdict. A stability score of 0.203 and aggregation propensity of 0.193 suggest the modified sequence is not flagged for gross stability failures, and the half-life estimate remains long — consistent with the trans-3-hexenoyl cap's known protective effect. BBB penetration of 0.011 is expected and irrelevant for a GH-axis peripheral peptide. These heuristics confirm the Aib substitution does not appear to introduce catastrophic sequence-level liabilities, but they cannot substitute for structural confidence.

The core difficulty of this fold is that a 44-residue peptide complexed with a class B GPCR is at the upper edge of what current single-run structure prediction tools can reliably resolve. The GHRHR lacks a published high-resolution co-crystal structure with tesamorelin or native GHRH that would provide a template for confident prediction. The low pLDDT likely reflects genuine model uncertainty about the peptide's conformation in the bound state rather than a signal that the modification is structurally disruptive — but these two interpretations are indistinguishable with the data in hand. This is a tool limitation, not a biological verdict.

From a medicinal chemistry perspective, the Gln-8 → Aib substitution remains a mechanistically plausible hypothesis. The key unresolved trade-off — whether the loss of Gln's hydrogen-bonding capacity to GHRHR outweighs the gain in helical pre-organization — cannot be adjudicated computationally at this time. The literature is entirely silent on position-8 SAR in tesamorelin or close analogues, and no high-resolution structural data on the tesamorelin–GHRHR complex exists to anchor computational models. Wet-lab approaches, particularly circular dichroism to confirm helicity gains and receptor binding assays against GHRHR, are the appropriate next steps if this hypothesis is to be advanced.

In the broader lab narrative, Fold №13 joins Fold №2 (Sermorelin D-Ala-2) as a case where a mechanistically sound hypothesis applied to a longer or more complex peptide returns a low-confidence structural prediction. Both returned pLDDT ≈ 0.49 and uninformative ipTM values. This pattern suggests a systematic limitation: the current toolchain may not be well-suited to resolving mid-helix non-proteogenic substitutions in 40+ residue peptides complexed with class B GPCRs without either ensemble prediction or a high-quality structural template. The positive contrast remains Fold №4 (Ipamorelin, 5-mer, REFINED), where a shorter peptide with a simpler receptor context yielded a pLDDT of 0.80 and a confident verdict.

RESEARCH BRIEF

FOLD №13 — TESAMORELIN GLN-8 → AIB HELIX STABILIZATION

Verdict: DISCARDED | Peptide: Tesamorelin | Class: Performance | Target: GHRHR (Q02643)

MECHANISM OF ACTION (BACKGROUND)

Tesamorelin is a synthetic 44-residue analogue of human GHRH(1-44), modified at the N-terminus with a trans-3-hexenoyl group to protect against DPP-IV cleavage at the Tyr-1/Ala-2 bond. It is the only FDA-approved GHRH analogue, indicated for HIV-associated lipodystrophy, and acts by binding to the growth hormone-releasing hormone receptor (GHRHR, UniProt Q02643) on somatotroph cells of the anterior pituitary, stimulating pulsatile GH secretion. Downstream GH and IGF-1 signaling drives lipolysis, preferentially reducing visceral adipose tissue. Efficacy has been demonstrated across multiple Phase III trials and 52-week extension studies, including on modern integrase inhibitor-based ART (Russo et al. 2024), confirming the robustness of the GHRHR pathway as a pharmacological target.

The molecular basis of GHRHR engagement depends critically on the amphipathic α-helical conformation of residues approximately 1–13 of GHRH/tesamorelin. This N-terminal helix presents a hydrophobic face for receptor contact and a solvent-exposed face of polar residues. Disruption of helical geometry in this region attenuates receptor potency. The trans-3-hexenoyl cap provides both proteolytic protection and potential conformational stabilization at the N-terminus — but the mid-helix region (residues 4–13) remains flexible in solution and may adopt the bioactive conformation only upon receptor encounter, incurring an entropic binding penalty.

MODIFICATION HYPOTHESIS (WHAT WE TESTED)

This distillation tested whether substituting Gln-8 with α-aminoisobutyric acid (Aib) could pre-organize residues 1–13 of Tesamorelin into the bioactive α-helical conformation, reducing the entropic cost of GHRHR binding and potentially adding a second axis of proteolytic protection via conformational rigidity at a mid-helix site.

Modified sequence: YADAIFT**(Aib)**SYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL

The rationale was multi-layered: - Gln-8 sits on the **solvent-exposed face** of the N-terminal helix and is not known to make direct receptor contacts, making it a

structurally permissive site for substitution. - Aib's **geminal dimethyl group** on the α-carbon sterically enforces φ/ψ dihedral angles within the canonical α-helical region (approximately −60°/−45°), and its helix-inducing properties are extensively validated in peptide chemistry literature. - Aib-type chemistry has direct precedent in GH-axis peptides: **Fold №4 (Ipamorelin N-Me-Aib, REFINED, pLDDT 0.80)** demonstrated that Aib-class substitutions are compatible with GH secretagogue receptor engagement and yield high-confidence predicted structures in a related peptide. - This strategy is **chemically and positionally distinct** from the DPP-IV-blocking N-terminal approaches tested in Fold №2 (Sermorelin D-Ala-2, DISCARDED) and is orthogonal to the trans-3-hexenoyl cap already present in tesamorelin — targeting conformational rigidity rather than exopeptidase blockade.

The predicted outcome was pLDDT >0.85 across residues 4–13 with preserved overall fold geometry.

WHY THE PREDICTION WAS UNINFORMATIVE (TECHNICAL ANALYSIS OF THE METRICS)

The structure prediction returned metrics that preclude any confident interpretation:

Metric	Value	Threshold for confidence
Peptide pLDDT	**0.491**	>0.70 for moderate, >0.85 for high
pTM	0.441	>0.60 for moderate confidence
ipTM	**0.340**	>0.60 for reliable interface
Chai-1 agreement	None	—
Boltz-2 affinity	Not produced	—

The pLDDT of 0.491 indicates substantial uncertainty in backbone placement throughout the 44-residue analogue — the model cannot confidently assign secondary structure elements, including the very N-terminal helix that is the subject of the hypothesis. The ipTM of 0.34 means the predicted peptide-receptor docking pose is not reliably resolved; the interface geometry cannot be trusted. The absence of Chai-1 agreement and Boltz-2 affinity values removes the secondary confidence checks that would ordinarily triangulate a weak primary signal.

Notably, this pLDDT value is nearly identical to **Fold №2 (Sermorelin D-Ala-2, pLDDT 0.49, DISCARDED)** — suggesting a systemic pattern rather than a modification-specific failure. Both folds involved non-proteogenic substitutions in longer GHRH-class peptides complexed with GHRHR. The contrast with **Fold №4 (Ipamorelin, pLDDT 0.80, REFINED)** — a shorter pentapeptide GHS targeting GHSR — is instructive: peptide length and template availability appear to substantially influence prediction reliability in this receptor class.

The heuristic sequence-based properties (aggregation propensity 0.193, stability score 0.203, long half-life estimate) suggest no gross sequence-level liabilities were introduced by the Aib substitution, but these cannot substitute for structural confidence.

Critical distinction: A pLDDT of 0.49 does not mean the modification is structurally disruptive. It means the model is uncertain — the two interpretations (modification causes structural disruption vs. model lacks template fidelity for this complex) are indistinguishable with the data in hand. The GHRHR lacks a published high-resolution co-crystal structure with tesamorelin or native GHRH, depriving the prediction model of the template quality needed to confidently resolve a 44-residue peptide at a class B GPCR interface.

WHAT THIS TELLS US (NEGATIVE RESULTS ARE DATA)

Fold №13 does not falsify the Gln-8 → Aib hypothesis. It establishes that **this hypothesis cannot be evaluated with single-run structure prediction at the current state of the toolchain** for this peptide-receptor system. That is a meaningful, if frustrating, result.

Specifically, this fold rules out the possibility of obtaining a high-confidence computational endorsement of the helix-preorganization strategy for tesamorelin using the current pipeline. It also narrows the design space: any computational approach to tesamorelin SAR at internal helix positions will require either (a) a high-resolution structural template for the tesamorelin–GHRHR complex, (b) ensemble prediction methods that can average over conformational uncertainty, or (c) fragment-based approaches that model the N-terminal helix independently before docking.

The key mechanistic trade-off — whether Aib's helix-inducing effect outweighs the loss of Gln-8's hydrogen-bonding capacity to GHRHR residues — remains entirely unresolved. The literature is silent on position-8 SAR in any GHRH analogue, meaning this is a genuine knowledge gap rather than a resolved question with a negative answer.

In the context of the lab's emerging pattern: Folds №2 and №13 both returned pLDDT ≈ 0.49 on GHRHR-targeting peptides ≥29 residues. Fold №4 (Ipamorelin, 5-mer, GHSR target) returned pLDDT 0.80. This pattern suggests the lab should either (1) focus computational GHRH work on shorter, truncated analogues (GHRH(1-17) or GHRH(1-29) fragments) where prediction confidence may be recoverable, or (2) escalate to ensemble/multi-model approaches before investing further single-run credits on full-length tesamorelin variants.

ALTERNATIVE HYPOTHESES TO TEST (AVOID THE FAILURE MODE)

Computational approaches to recover signal: - **Truncated analogue:** Test Aib-8 in a GHRH(1-17) or GHRH(1-29) framework — shorter peptides yield higher pLDDT in this receptor class, and SAR mapping in truncated analogues is standard practice in the GHRH literature. - **Ensemble prediction:** Run 5–10 predictions with different random seeds and report mean/SD pLDDT and ipTM; ensemble agreement would provide a confidence interval on the structural uncertainty. - **Fragment modeling:** Model the N-terminal helix (residues 1–13) in isolation to assess whether Aib-8 measurably increases helical propensity before attempting full complex docking. - **Alternative helix-inducing substitutions at position 8:** α-methyl-phenylalanine (MePhe) or L-α-methyl-glutamine could probe whether steric reinforcement at the α-carbon is the operative variable while partially preserving side-chain character.

Wet-lab validation path (if hypothesis is to be advanced): - **Circular dichroism (CD) spectroscopy:** Direct measurement of helical content in the Aib-8 analogue vs. native tesamorelin — the most direct test of the helix-preorganization hypothesis. - **GHRHR binding assay (competitive radioligand or TR-FRET):** Measures affinity change independently of structural confidence; would resolve the Aib vs. Gln trade-off question computationally intractable here. - **Plasma stability assay:** Incubation in human plasma with HPLC/MS quantification would determine whether mid-helix Aib confers proteolytic protection beyond what the trans-3-hexenoyl cap already achieves. - **NMR (solution structure):** Would directly confirm or deny helical preorganization in the N-terminal segment — gold standard for the conformational hypothesis.

SEQUENCES

NATIVE

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YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL
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MODIFIED

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YADAIFT(Aib)SYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL
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CAVEATS

- in silico prediction only — requires wet lab validation

- single-run prediction (not ensembled) — pLDDT 0.49 reflects model uncertainty, not confirmed structural disruption
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- no high-resolution tesamorelin–GHRHR co-crystal structure exists to template the prediction; low confidence may be a tool limitation rather than a biological signal
- heuristic property estimates (aggregation, stability, half-life, BBB) are sequence-based approximations only — not experimental measurements
- loss of Gln-8 hydrogen-bonding capacity to GHRHR cannot be assessed without structural data; binding affinity trade-off is unresolved
- Aib cannot be represented by standard amino acid force fields in all structure prediction pipelines — non-proteogenic residue handling may introduce additional model uncertainty

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 3quX4H7mksnKNpV7Yu5sn4dDDf6UXSaV6DnDQMcy3pcaL5gYQwo68vA6i2eycCyTFeVADq2GUFZC8RJZFSADqmtr
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