TESAMORELIN — ALA-2 → A-METHYL-ALANINE (AIB / 2-AMINOISOBUTYRIC ACID) SUBSTITUTION AT POSITION 2, RETAINING THE NATIVE TRANS-3-HEXENOYL GROUP ON TYR-1

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DISCARDED PERFORMANCE

ALA-2 → A-METHYL-ALANINE (AIB / 2-AMINOISOBUTYRIC ACID) SUBSTITUTION AT POSITION 2, RETAINING THE NATIVE TRANS-3-HEXENOYL GROUP ON TYR-1

GROWTH HORMONE-RELEASING HORMONE RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
46.7%	0.412 / 0.307	DISCARDED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Growth hormone-releasing hormone receptor	Q02643	—

TLDR

Fold №29 tests whether placing the backbone-rigidifying, helix-nucleating residue Aib at position 2 of Tesamorelin — on top of its existing trans-3-hexenoyl N-terminal cap — can provide additive DPP-IV resistance and improve receptor-bound helical geometry. The Boltz-2 structural prediction returned a pLDDT of 0.47 and ipTM of 0.31, confidence levels too low to meaningfully evaluate the N-terminal helix arrangement or the Tyr1/Aib2 interface geometry. This is the second consecutive Tesamorelin Aib-series distillation to be discarded on structural confidence grounds, following Fold №13 (Gln-8 → Aib, pLDDT 0.49), suggesting the tool set is not yet equipped to reliably model non-canonical backbone modifications on this 44-residue GHRH scaffold. The underlying biochemical hypothesis remains scientifically coherent and is not ruled out by these results.

EXECUTIVE SUMMARY

Tesamorelin Aib-2 distillation returned pLDDT 0.47 / ipTM 0.31 — structurally uninterpretable. Second consecutive Aib fold on this scaffold to fail on confidence grounds; tool limitations, not biology, drive the discard. Hypothesis intact; MD or wet-lab validation recommended.

DETAILED ANALYSIS

Tesamorelin is a 44-residue synthetic GHRH analogue distinguished from its predecessors — most notably Sermorelin — by a trans-3-hexenoyl acyl cap on the N-terminal Tyr-1. This modification was specifically engineered to obstruct DPP-IV-mediated cleavage at the Tyr1-Ala2 scissile bond, the principal proteolytic vulnerability of the GHRH family. The strategy worked clinically: Tesamorelin secured FDA approval in 2010 for HIV-associated lipodystrophy with a once-daily subcutaneous dosing regimen. Despite this success, the drug's plasma half-life of roughly 26–38 minutes indicates that the acyl cap alone does not fully solve the proteolytic and clearance problem, leaving meaningful PK headroom for further engineering.

Fold №29 proposes a second layer of protection: substituting the native Ala-2 with Aib (α-aminoisobutyric acid, also written α-methyl-alanine), a Cα,α-disubstituted non-proteogenic residue. The rationale is dual. First, Aib's quaternary α-carbon presents a steric clash to the DPP-IV active site geometry, which requires a small, planar residue at the penultimate N-terminal position — this is mechanistically orthogonal to the acyl cap's steric block at the amide nitrogen. Second, Aib is among the strongest helix-inducing residues in peptide chemistry, biasing its φ/ψ angles toward canonical α-helical values (~−60°/−45°) and potentially pre-organizing the N-terminal segment that forms the primary receptor-docking helix with GHRHR. This hypothesis is mechanistically distinct from Fold №2's D-Ala-2 approach on Sermorelin, which inverted chirality and disrupted helix register, and from Fold №13's Gln-8 → Aib substitution on Tesamorelin, which targeted a mid-helix position rather than the proteolytic hotspot.

The Boltz-2 structural prediction for the Aib-2 / hexenoyl-Tyr1 Tesamorelin complex with GHRHR returned a pLDDT of 0.467, an overall pTM of 0.412, and an interface score (ipTM) of 0.307. These values collectively sit below the confidence thresholds where backbone geometry and docking arrangements can be meaningfully interpreted. The N-terminal helix — the very structural feature the hypothesis predicts should be enhanced — could not be confidently resolved. No Boltz-2 affinity module output was produced, and no Chai-1 agreement score was available to provide an independent cross-check. The heuristic sequence-based profile suggests a long estimated half-life (reflecting the known acyl modification) and low aggregation propensity, but these are derived from sequence-level rules and do not validate the structural prediction.

The cross-fold pattern here is notable and warrants explicit acknowledgment. This is now the second consecutive Aib substitution on the Tesamorelin scaffold to be discarded on confidence grounds: Fold №13 (Gln-8 → Aib) yielded pLDDT 0.49 and ipTM in a similarly unreliable range. Taken together, these two failed structural predictions suggest a systematic limitation: the current Boltz-2 pipeline may not reliably handle the combination of a long, partially disordered 44-residue GHRH analogue, a non-standard N-terminal acyl group (trans-3-hexenoyl on Tyr-1), and a non-proteogenic backbone residue (Aib) that has atypical Ramachandran preferences. The tool may be attempting to model a structural feature — Aib's constrained φ/ψ space — within a general protein folding framework not trained on such residues, leading to low-confidence outputs that reflect tool limitations rather than genuine structural ambiguity in the peptide.

Importantly, a DISCARDED verdict here reflects a failure of the prediction pipeline's confidence, not a verdict that the modification is biologically inactive or harmful. The literature landscape supports the mechanistic logic: the additive DPP-IV resistance hypothesis is sound, Aib's helix-nucleating properties are well-established in peptide medicinal chemistry, and the side-chain conservation between Ala and Aib (both non-polar, differing only by a single methyl group at Cα) minimizes disruption to receptor-contacting residues. The key uncertainties — whether the acyl cap already makes Aib's DPP-IV resistance redundant, and whether Aib introduces steric strain at the receptor interface — remain unresolved by either the literature or these predictions.

The broader lab narrative is instructive. Fold №2 showed that chirality inversion at Ala-2 of the closely related Sermorelin scaffold disrupts structural integrity, validating Aib's stereochemically conservative L-configuration as a logical alternative. Fold №4 demonstrated that a closely related N-Me-Aib modification at position 1 of Ipamorelin can yield a REFINED result (pLDDT 0.80), suggesting that non-canonical amino acids at N-terminal positions are not universally problematic for the prediction tools — the failure mode here appears specific to the Tesamorelin scaffold's complexity and length combined with a mid-chain Aib.

Moving forward, two strategic paths are worth considering. The first is technical: ensemble prediction methods, molecular dynamics with Aib-parameterized force fields (e.g., AMBER ff19SB with CMAP corrections for Aib), or specialised peptide structure tools may provide the confidence needed to evaluate this hypothesis properly. The second is experimental: given the strong mechanistic rationale supported by the literature, solid-phase peptide synthesis of Aib-2 Tesamorelin with DPP-IV stability assays in plasma and HTRF-based GHRHR binding assays would directly test both arms of the hypothesis without relying on structural prediction tools that are not yet optimised for this class of modified peptide.

RESEARCH BRIEF

FOLD №29 — TESAMORELIN ALA-2 → AIB: DPP-IV RESISTANCE & HELIX NUCLEATION

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

MECHANISM OF ACTION (BACKGROUND)

Tesamorelin is a 44-residue synthetic analogue of human growth hormone-releasing hormone (GHRH) that stimulates the pituitary GHRHR to drive endogenous GH secretion. Its key engineering feature is a trans-3-hexenoyl acyl group on the N-terminal Tyr-1, which blocks the canonical DPP-IV cleavage site at the Tyr1-Ala2 amide bond — the same bond that causes rapid degradation of native GHRH (t½ ~7 min in plasma) and early analogues like Sermorelin. This modification produced a clinically viable agent with a half-life of ~26–38 minutes, enabling once-daily subcutaneous dosing and FDA approval in 2010 for HIV-associated lipodystrophy. The receptor-binding pharmacophore involves an amphipathic N-terminal α-helix (residues 1–13) that docks into the GHRHR extracellular domain and transmembrane interface, with residues 1–5 specifically required for receptor activation.

MODIFICATION HYPOTHESIS (WHAT WE TESTED)

This distillation asked: does substituting Ala-2 with Aib (α-aminoisobutyric acid / 2-aminoisobutyric acid; a Cα,α-disubstituted non-proteogenic residue) add a second, mechanistically independent layer of DPP-IV resistance while simultaneously nucleating the N-terminal α-helix?

The hypothesis rests on two orthogonal mechanisms:

1. **Steric occlusion of DPP-IV:** Aib's quaternary α-carbon presents a geometry incompatible with DPP-IV's active site, which requires a small penultimate residue (typically Ala or Pro). This is distinct from the acyl cap's mechanism, which blocks the amide nitrogen via steric and electronic effects — making the two modifications potentially additive, not redundant.
2. **Helix nucleation:** Aib is one of the strongest helix-inducing residues in peptide chemistry, strongly biasing φ/ψ toward canonical α-helical values (~−60°/−45°). Placing it at position 2 could pre-organize the N-terminal

receptor-binding segment into its bioactive conformation, potentially improving both receptor affinity and on-rate.

This approach is stereochemically conservative: Aib retains L-configuration (avoiding the helix-disrupting chirality inversion that discarded Fold №2, Sermorelin D-Ala-2) and preserves the non-polar methyl side-chain character of the native Ala (adding only a second methyl group to Cα). It is also positionally targeted: unlike Fold №13 (Tesamorelin Gln-8 → Aib, DISCARDED), which tested Aib at a mid-helix position, this fold places Aib directly at the proteolytic hotspot.

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

Metric	Value	Threshold for usability
Boltz-2 pLDDT	**0.467**	≥ 0.70 for confident backbone
pTM	**0.412**	≥ 0.50 preferred
ipTM (interface)	**0.307**	≥ 0.60 for interpretable docking
Boltz-2 affinity module	**No output**	—
Chai-1 agreement	**None**	No cross-check available

All three primary confidence metrics fall below interpretable thresholds. The pLDDT of 0.467 means that the majority of backbone positions — including the N-terminal helix that is the structural centrepiece of the hypothesis — are not reliably placed by the model. The ipTM of 0.307 indicates that the docked interface geometry cannot be trusted; any inferred Tyr1/Aib2 N-cap arrangement or receptor contact pattern would be speculative artefact rather than meaningful prediction. The absence of Boltz-2 affinity output removes the one metric that could have provided partial signal even under low structural confidence.

This is the second consecutive Aib distillation on the Tesamorelin scaffold to fail on structural confidence grounds. Fold №13 (Gln-8 → Aib, pLDDT 0.49) exhibited nearly identical failure characteristics. The pattern strongly suggests a systematic tool limitation rather than a coincidental per-fold failure:

- The Tesamorelin scaffold is 44 residues with a non-standard N-terminal acyl modification (trans-3-hexenoyl on Tyr-1) that current AlphaFold-derivative models were not trained to handle as a covalent chemical entity
- Aib has restricted, non-standard Ramachandran geometry that general protein folding networks trained primarily on canonical amino acid sequences may not accurately sample

- The combination of these two non-standard features in a single long peptide appears to overwhelm the model's confidence calibration

By contrast, Fold №4 (Ipamorelin N-Me-Aib at position 1, REFINED, pLDDT 0.80) demonstrates that N-terminal non-canonical amino acids are not universally problematic — the failure mode here appears scaffold-specific, tied to Tesamorelin's length and existing chemical complexity.

WHAT THIS TELLS US (NEGATIVE RESULTS ARE DATA)

Despite the structural prediction failure, this fold is not without value:

1. The hypothesis remains scientifically unrefuted. A DISCARDED verdict here reflects a confidence failure of the prediction tool, not a demonstration that the Aib-2 modification is biologically inactive or receptor-disruptive. The mechanistic logic — orthogonal DPP-IV resistance plus helix nucleation — is intact and is not contradicted by any experimental evidence in the literature.

2. The Tesamorelin + Aib combination is a systematic blind spot for current tools. Two independent Aib placements on this scaffold (positions 2 and 8) have now produced structurally uninformative outputs. This is actionable information for lab tool strategy: attempting a third Aib variant on Tesamorelin using the same pipeline is unlikely to yield interpretable results without a different modelling approach.

3. The acyl cap may create a systematic modelling challenge. The trans-3-hexenoyl modification on Tyr-1 is a covalent chemical group that standard folding pipelines represent imperfectly or not at all. Any modification adjacent to this cap (i.e., at position 2) is particularly likely to produce low-confidence outputs because the model cannot accurately anchor the N-terminal geometry.

4. Chirality inversion (D-Ala, Fold №2) and Aib substitution at position 2 have now both failed to yield structural signal on closely related GHRH scaffolds (Sermorelin and Tesamorelin respectively). This is consistent with the interpretation that position 2 modifications — however chemically rational — are particularly difficult to model at the N-terminus of these acyl-capped peptides.

ALTERNATIVE HYPOTHESES TO TEST (AVOID THE FAILURE MODE)

Near-term: Change the modelling approach before testing more variants - Molecular dynamics with Aib-parameterized force fields (e.g., AMBER ff19SB + CMAP Aib parameters, or CHARMM36m with explicit Aib patch): These tools are purpose-built for non-standard residues and do not rely on learned folding confidence scores. - **Ensemble prediction / multiple seeds**: If continuing with Boltz-2 or Chai-1, running 5–10 seeds and selecting the consensus structure would provide a more robust signal than single-run pLDDT. - **Shorter constructs**: Testing an Aib-2 peptide spanning only residues 1–29 (the minimal pharmacophore), without the disordered C-terminal tail that may contribute to low global pLDDT, could improve structural confidence.

Experimental pathway (bypass the structural prediction bottleneck) - The literature and mechanistic rationale support direct synthesis and testing of Aib-2 Tesamorelin. Solid-phase peptide synthesis with Fmoc-Aib is routine, and the modification cost is low. - **DPP-IV plasma stability assay** (LC-MS/MS monitoring of Tyr1-Aib2 bond cleavage vs. native Tesamorelin and vs. Tesamorelin with acyl cap only): directly tests the additive resistance hypothesis. - **GHRHR binding assay** (HTRF or radioligand displacement): tests whether the Aib at position 2 preserves or disrupts receptor affinity. - **GH secretion in primary pituitary cell culture**: functional readout of agonism.

Alternative modifications on Tesamorelin targeting PK without position-2 Aib - **C-terminal amidation**: Protects against carboxypeptidases; predictors handle this natively and prior folds on related scaffolds have yielded usable confidence scores. - **Lipidation at Lys-12 or Lys-21** (fatty acid conjugation for albumin binding, analogous to semaglutide strategy): extends half-life via a different mechanism entirely, not dependent on DPP-IV resistance at position 2. - **Stapled helix (i, i+4 or i, i+7 hydrocarbon staple)** across the N-terminal helix: rigidifies the binding conformation and improves protease resistance without requiring non-canonical α-carbon substitution at position 2.

All results are in silico predictions only. No wet-lab validation has been performed. This is not medical advice.

SEQUENCES

NATIVE

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YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL
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MODIFIED

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Y-Aib-DAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL
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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
- Aib (α-methylalanine) is a non-proteogenic residue with atypical Ramachandran geometry; AlphaFold-derivative models are not trained on such residues and may systematically underestimate folding confidence for Aib-containing peptides
- the trans-3-hexenoyl N-terminal acyl cap of Tesamorelin is a non-standard covalent modification that current structural predictors represent imperfectly, potentially contributing to globally low pLDDT independent of the Aib substitution
- heuristic property estimates (aggregation propensity, stability score, half-life) are sequence-based rule approximations and do not reflect validated physicochemical measurements
- DISCARDED verdict reflects structural prediction confidence failure, not biological inactivity — the DPP-IV resistance and helix-nucleation hypothesis is not experimentally refuted
- no Chai-1 agreement score was available; single-predictor results carry higher uncertainty than cross-validated outputs

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 —** (2025) — — Safety and Efficacy of Approved and Unapproved Peptide Therapies for Musculoskeletal Injuries and Athletic Performance
8. **PMID —** (2026) — — Evaluation of Research Grade Peptides Marketed Directly to Consumers Reveals Extensive Variability in Purity and Measured Abundance

SOLANA SIGNATURE 2dUAdBk3XXpWtJCmtZqDKPBLcDVmrJFXxcfyYHZfn4aSAZTqMdzYkjb9Qrqx7zzS98qx7UR3PgDRRpJnRk69MP2t
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