DISTILLATION №69 · ALEMBIC LABS

SERMORELIN — ALA-2 → AIB (A-AMINOISOBUTYRIC ACID) SINGLE SUBSTITUTION AT POSITION 2

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DISCARDED | PERFORMANCE

ALA-2 → AIB (A-AMINOISOBUTYRIC ACID) SINGLE SUBSTITUTION AT POSITION 2

GROWTH HORMONE-RELEASING HORMONE RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
48.5%	0.462 / 0.420	DISCARDED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Growth hormone-releasing hormone receptor	Q02643	—

TLDR

Fold №69 tested whether replacing Ala-2 of Sermorelin with α-aminoisobutyric acid (Aib) — the canonical DPP-IV-resistant Ala mimetic used in semaglutide and tirzepatide — could extend plasma half-life while preserving GHRHR binding. The structural prediction returned a pLDDT of 0.48 and ipTM of 0.42, confidence levels too low to adjudicate whether the N-terminal Aib-2 occupies the same binding geometry as native Ala-2. This fold is DISCARDED as a tool-limit failure, not a biological invalidation: the hypothesis remains chemically sound and literature-supported. It joins a consistent pattern of sub-threshold confidence across all seven prior Sermorelin and Tesamorelin folds in this lab.

EXECUTIVE SUMMARY

Aib-2 Sermorelin: pLDDT 0.48, ipTM 0.42 — tool-limit discard, not biological disproof. GHRHR resists confident prediction across all six lab folds. DPP-IV resistance hypothesis remains chemically sound; wet-lab validation required.

DETAILED ANALYSIS

Sermorelin (YADAIFTNSYRKVLGQLSARKLLQDIMSR) is the shortest GHRH fragment retaining full biological activity at the GHRHR, but its clinical utility is curtailed by a plasma half-life of under 15 minutes. The primary enzymatic culprit is DPP-IV, which cleaves the Tyr1–Ala2 bond to yield the inactive [3-29] fragment — a metabolite confirmed in anti-doping analytical literature and detected in biological matrices under serum protease challenge. This vulnerability is well-established, and the clinical field has already validated N-terminal engineering as the solution: Tesamorelin's trans-3-hexenoic acid N-cap and CJC-1295's DAC conjugation both extend GHRH-class half-life while preserving GHRHR agonism, confirming the receptor's tolerance for position-1/2 modification.

The Aib substitution strategy proposed here is among the most chemically conservative tools available for solving the DPP-IV problem. α-Aminoisobutyric acid (α-methyl alanine) is an achiral, α,α-disubstituted residue whose gem-dimethyl Cα sterically occludes the DPP-IV catalytic pocket while retaining a pseudo-L backbone orientation. Unlike the D-Ala substitution explored in Fold #2 — which inverts backbone stereochemistry and risks disrupting the receptor interaction geometry — Aib preserves the L-configured backbone face seen by the GHRHR extracellular domain. Furthermore, Aib is a potent helix-inducing residue, meaning it could subtly bias the N-terminal cap toward the α-helical geometry that dominates GHRHR engagement across the mid-peptide and C-terminal segments. The theoretical case for [Aib2]-Sermorelin is therefore stronger than for [D-Ala2]-Sermorelin, making it unfortunate that both folds collide with the same tool-limit ceiling.

The structural prediction was run on the modified sequence Y-Aib-DAIFTNSYRKVLGQLSARKLLQDIMSR against the GHRHR extracellular domain (UniProt Q02643). The returned pLDDT of 0.48 and ipTM of 0.42 are essentially identical to the values seen across every prior Sermorelin and Tesamorelin fold in this lab: Fold #53 (hexenoyl N-cap, pLDDT 0.49), Fold #42 (lactam staple, pLDDT 0.50), Fold #2 (D-Ala2, pLDDT 0.49), and Fold #60 (Tesamorelin truncation, pLDDT 0.48). The emergence of a consistent ~0.48–0.50 pLDDT floor across structurally diverse modifications on this target is itself informative: it suggests the GHRHR ECD is a genuinely difficult system for current AlphaFold-class predictors, likely because Class B GPCR extracellular domains sample large conformational ensembles and lack sufficient co-complex training data to produce high-confidence complex structures.

The heuristic peptide profile is unremarkable and consistent with a Sermorelin scaffold: aggregation propensity of 0.164 (low), stability score of 0.319, and a moderate-to-long heuristic half-life estimate (1–6 hours, though this is a sequence-based heuristic and does not model enzymatic cleavage). Notably, the heuristic half-life estimate reflects the Aib2 modification's expected DPP-IV resistance, but cannot be taken as a predicted in vivo half-life — real-world PK would also reflect

renal filtration (Sermorelin is ~3,357 Da, near the glomerular filtration threshold) and other protease exposures not captured by these estimates.

The absence of Chai-1 agreement data and Boltz-2 affinity values means no orthogonal signal is available to rescue the verdict. With a single-run pLDDT below 0.55 and ipTM below 0.50, the model cannot reliably distinguish whether Aib-2 occupies the same backbone position as native Ala-2 or whether the N-terminal cap has shifted off the receptor interface entirely. The single-residue Ala→Aib substitution is too subtle a chemical perturbation to emerge from background noise at this confidence level.

This result does not disprove the hypothesis. The DPP-IV resistance rationale for Aib at position 2 is validated in incretin pharmacology at the level of marketed drugs (semaglutide, tirzepatide) and is mechanistically sound for the GHRH scaffold. The absence of a GHRHR co-crystal structure with any Aib-containing peptide is a gap in the field, not evidence that such binding cannot occur. The question this fold asked — does Aib-2 preserve GHRHR binding geometry? — simply cannot be answered by current in silico tools at this confidence level.

Looking across the full Sermorelin/Tesamorelin campaign in this lab (Folds #2, #42, #50, #53, #60, #69), a clear pattern emerges: no modification to the Sermorelin/Tesamorelin scaffold has achieved an adjudicable structural prediction against the GHRHR ECD. The consistent sub-threshold confidence suggests the limiting factor is the target representation, not the peptide modifications themselves. Future work on this scaffold should prioritize wet-lab approaches — binding assays, DPP-IV stability assays, and ideally cryo-EM or X-ray crystallography of the [Aib2]-Sermorelin/GHRHR complex — over further in silico permutations against this receptor until a better-resolved training structure becomes available.

RESEARCH BRIEF

FOLD №69 — SERMORELIN [AIB2]: DPP-IV RESISTANCE VIA A-AMINOISOBUTYRIC ACID

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

TLDR

Fold №69 is **DISCARDED** due to a **tool-limit failure**: the structural predictor could not achieve sufficient confidence (pLDDT 0.48, ipTM 0.42) to adjudicate whether replacing Ala-2 of Sermorelin with α-aminoisobutyric acid (Aib) preserves GHRHR

binding geometry. This is not a biological invalidation — the DPP-IV resistance hypothesis for [Aib2]-Sermorelin is chemically sound and extrapolated from validated incretin pharmacology. The GHRHR extracellular domain has consistently resisted confident structural prediction across all seven Sermorelin/Tesamorelin folds in this lab, suggesting the bottleneck is the **target representation in current predictors**, not the peptide modifications themselves.

WHAT WE TRIED

We hypothesized that substituting the native Ala at position 2 of Sermorelin with α-aminoisobutyric acid (Aib, α-methyl alanine) would block DPP-IV cleavage at the Tyr1–Ala2 bond — the confirmed primary degradation pathway — without disrupting GHRHR binding or activation. The modified sequence, Y-**Aib**-DAIFTNSYRKVLGQLSARKLLQDIMSR, was selected because Aib is the canonical DPP-IV-resistant Ala mimetic at position 2, validated in semaglutide and tirzepatide at the analogous GLP-1 scaffold position.

The mechanistic case for Aib over the previously tested D-Ala (Fold #2, also DISCARDED) is that Aib's gem-dimethyl Cα provides steric occlusion of DPP-IV's catalytic pocket while maintaining a pseudo-L backbone orientation — retaining the receptor interaction geometry that D-Ala inversion risks disrupting. Aib is also a strong helix-inducing residue, raising the possibility that position-2 substitution could subtly enhance the helical pre-organization of the N-terminal cap, which is the activation domain for GHRHR. This fold was designed as the most conservative, chemically defensible test of DPP-IV resistance on this scaffold.

WHY IT WAS DISCARDED

The structural prediction returned: - **pLDDT: 0.48** — below the 0.55 threshold required for meaningful interface analysis - **ipTM: 0.42** — indicating the model could not confidently resolve the peptide–receptor interface - **Chai-1 agreement: not available** - **Boltz-2 affinity: not available**

At this confidence level, the model cannot distinguish whether Aib-2 occupies the same backbone position as native Ala-2 or whether the N-terminal segment has adopted an off-target conformation. The single-residue Ala→Aib substitution — a subtle change adding only two methyl groups at Cα — is chemically too small to emerge from prediction noise when the baseline complex confidence is already sub-threshold.

This result is consistent with a now well-established pattern across the entire Sermorelin/Tesamorelin campaign in this lab:

Fold	Modification	pLDDT	Verdict
#2	Ala-2 → D-Ala	0.49	DISCARDED
#42	Lys21–Asp25 lactam staple	0.50	DISCARDED
#50	Val13/Leu17 hydrocarbon staple (Tesamorelin)	0.46	DISCARDED
#53	trans-3-Hexenoyl N-cap + C-terminal amidation	0.49	DISCARDED
#60	Tesamorelin C-terminal truncation to GHRH(1-29)	0.48	DISCARDED
#69	**Ala-2 → Aib**	**0.48**	**DISCARDED**

The consistency of the ~0.48–0.50 pLDDT floor across structurally diverse modifications — N-terminal caps, staples, stereochemical inversions, truncations, and now Aib substitution — points to a **target-level limitation**: the GHRHR extracellular domain is poorly represented in current AlphaFold-class training data for co-complex prediction, likely reflecting the inherent conformational dynamics of Class B GPCR extracellular domains and the scarcity of high-resolution GHRHR co-crystal structures in the PDB.

WHAT THIS DOESN'T MEAN

DISCARDED is not "disproved." The DPP-IV resistance hypothesis for [Aib2]-Sermorelin rests on well-established incretin pharmacology: Aib at position 2 is validated across semaglutide, tirzepatide, and related GLP-1-class drugs as the canonical solution to DPP-IV susceptibility. The GHRHR's demonstrated tolerance for N-terminal modification — evidenced by the clinical activity of Tesamorelin (trans-3-hexenoic acid at Tyr1) and CJC-1295 (DAC conjugation) — further supports the plausibility that [Aib2]-Sermorelin could retain agonist activity. The gap in the literature is the absence of any direct SAR study testing Aib at position 2 of any GHRH scaffold, meaning this question is genuinely unanswered rather than answered negatively. Current in silico tools simply lack the resolution to adjudicate a single-residue backbone methylation against a dynamically challenging Class B GPCR extracellular domain. The biological hypothesis remains open.

WHAT WOULD ANSWER THE QUESTION

- **DPP-IV stability assay (in vitro):** Incubate [Aib2]-Sermorelin and native Sermorelin with purified DPP-IV or pooled human plasma; HPLC/LC-MS quantification of intact peptide vs. [3-29] cleavage product over 0–120 min. This is the most direct, rapid, and low-cost experiment to confirm the primary hypothesis and has precedent in GHRH anti-doping analytical literature (PMID:37688464).

- **GHRHR binding and cAMP functional assay:** Radioligand displacement or TR-FRET binding assay at GHRHR-expressing cells (e.g., HEK293 overexpression), followed by GH secretion assay in pituitary cell culture (rat primary or GH3 cells) to confirm preserved agonist potency and Emax. Standard pharmacological characterization for GHRH analogues.
- **Surface plasmon resonance (SPR) or ITC against GHRHR ECD:** Biophysical binding affinity measurement against recombinant GHRHR extracellular domain to directly confirm whether Aib-2 preserves the binding pose — the precise question the structural predictor could not answer.
- **Cryo-EM or X-ray crystallography of [Aib2]-Sermorelin/GHRHR complex:** Definitive structural answer; would also resolve the broader tool-limit failure seen across all six Sermorelin/Tesamorelin folds and provide a high-quality template for future in silico work on this target. Technically demanding but transformative for the field given the absence of any GHRHR co-crystal structure with a modified GHRH analogue.
- **Molecular dynamics (MD) simulation with explicit Aib parameters:** Classical MD or free-energy perturbation (FEP) with properly parameterized Aib force-field terms could provide binding free energy estimates not available from AlphaFold-class predictors, which handle non-canonical residue chemistry poorly.

RAW METRICS

Metric	Value
pLDDT	0.485
pTM	0.462
ipTM	0.420
Chai-1 agreement	Not available
Boltz-2 affinity	Not available
Predicted binding change	Not determinable
Aggregation propensity (heuristic)	0.164 (low)
Stability score (heuristic)	0.319
BBB penetration (heuristic)	0.05 (negligible)
Half-life estimate (heuristic)	Moderate-to-long (1–6 h)

Heuristic peptide profile values are sequence-based estimates only — not wet-lab measurements and not validated for non-canonical residues.

This distillation is an in silico prediction only. It is not medical advice, not a validated discovery, and not a recommendation for synthesis or administration. All predicted properties require experimental validation.

SEQUENCES

NATIVE

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YADAIFTNSYRKVLGQLSARKLLQDIMSR
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MODIFIED

```
Y-Aib-DAIFTNSYRKVLGQLSARKLLQDIMSR
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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 is a non-canonical residue not natively represented in AlphaFold-class training data — its backbone geometry may be approximated or misrepresented in the structural model
- heuristic half-life estimate (1–6 h) is sequence-based and does not model enzymatic cleavage or renal filtration — real PK would differ significantly
- GHRHR ECD has returned sub-threshold pLDDT (~0.48–0.50) across six structurally diverse modifications in this lab — confidence floor may be a target-level training data limitation, not a peptide property
- Chai-1 agreement and Boltz-2 affinity data were unavailable — no orthogonal signal to supplement the primary prediction

CITATIONS

1. **PMID —** (1999) — — Sermorelin: a review of its use in the diagnosis and treatment of children with idiopathic growth hormone deficiency.
2. **PMID —** (2006) — — Sermorelin: a better approach to management of adult-onset growth hormone insufficiency?
3. **PMID —** (2023) — — In-house standards derived from doping peptides: Enzymatic and serum stability and degradation profile of GHRP and GHRH-related peptides.

4. **PMID —** (2023) — — Cationic exchange SPE combined with triple quadrupole UHPLC-MS/MS for detection of GHRHs in urine samples.
5. **PMID —** (2026) — — Therapeutic Peptides in Orthopaedics: Applications, Challenges, and Future Directions.
6. **PMID —** (2020) — — Beyond the androgen receptor: the role of growth hormone secretagogues in the modern management of body composition in hypogonadal males.
7. **PMID —** (2026) — — Evaluation of Research Grade Peptides Marketed Directly to Consumers Reveals Extensive Variability in Purity and Measured Abundance
8. **PMID —** (2026) — — Safety and Efficacy of Approved and Unapproved Peptide Therapies for Musculoskeletal Injuries and Athletic Performance
9. **PMID —** (2025) — — Growth Hormone-Releasing Hormone Antagonists Increase Radiosensitivity in Non-Small Cell Lung Cancer Cells.
10. **PMID —** (2021) — — A potentially effective drug for patients with recurrent glioma: sermorelin.

SOLANA SIGNATURE 4wSvSZrn6PymW1bhVWWxFXyhw8XZubgzgfruPEYKo19Ar2ZT3pgJWfn4Yg857dEFXJ9WJyDchLNgSVjkQUknJ3d4
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