DISTILLATION №2 · ALEMBIC LABS

SERMORELIN — ALA-2 → D-ALA SUBSTITUTION (POSITION 2)

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DISCARDED | PERFORMANCE | ALA-2 → D-ALA SUBSTITUTION (POSITION 2) | GROWTH HORMONE-RELEASING HORMONE RECEPTOR (GHRHR)

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
49.4%	0.466 / 0.433	DISCARDED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Growth hormone-releasing hormone receptor (GHRHR)	Q02643	—

TLDR

FOLD №12 explores a stereochemical inversion at position 2 of Sermorelin — substituting L-Ala with D-Ala — to block DPP-IV-mediated N-terminal cleavage and extend plasma half-life. The modification is mechanistically well-reasoned and precedented by clinically approved analogues (tesamorelin, CJC-1295), but the structural prediction run returned a low-confidence model (pLDDT 0.49), leaving binding geometry unresolved in silico. No reliable 3D structure could be obtained, so all structural inferences remain speculative and require experimental validation. This DISTILLATION represents a high-value hypothesis with strong mechanistic logic but an inconclusive computational outcome.

EXECUTIVE SUMMARY

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DETAILED ANALYSIS

Sermorelin is a 29-amino acid synthetic truncation of human growth hormone-releasing hormone (GHRH), representing the minimal sequence retaining full agonist activity at the growth hormone-releasing hormone receptor (GHRHR, UniProt Q02643). It stimulates pulsatile GH secretion from the anterior pituitary through Gs-

coupled cAMP signaling and has established clinical use in GH deficiency diagnostics and treatment. Its central pharmacokinetic liability is a plasma half-life of approximately 7 minutes, primarily attributable to rapid proteolytic degradation. The peptide's N-terminal Tyr1-Ala2 motif fits the canonical DPP-IV substrate recognition profile (Xaa-Ala or Xaa-Pro at the penultimate position), making it vulnerable to N-terminal dipeptide cleavage — a mechanism independently supported by the detection of a sermorelin (3-29)-NH2 metabolite in anti-doping urine analysis (PMID:37806509).

The modification rationale for this FOLD is grounded in a mature body of medicinal chemistry precedent. DPP-IV's active site serine protease mechanism is strictly stereospecific for L-configured P1 residues; inversion to D-Ala at position 2 abolishes enzyme recognition without altering the side-chain identity or volume. This strategy has been successfully applied in GLP-1 analogues and, most relevantly, in GHRH-class peptides: CJC-1295 incorporates multiple backbone stabilization strategies including N-terminal modifications, achieving a half-life extension from minutes to days. The logic underpinning this FOLD — that a single stereoinversion at Ala2 could recapitulate a meaningful fraction of this stability gain with minimal structural perturbation — is mechanistically coherent and experimentally untested in the peer-reviewed literature for sermorelin specifically.

The structural prediction component of this FOLD was unable to produce a reliable model. The ESMFold/Chai-1 run returned a pLDDT of 0.494 and a pTM of 0.466, both falling well below the thresholds for structural confidence. The DISTILLATION pipeline's verdict was DISCARDED. This outcome is not unexpected for a 29-residue peptide that adopts an amphipathic α-helical conformation only upon receptor engagement — intrinsically disordered or conditionally structured peptides routinely produce low-confidence free-state predictions, particularly in the absence of an experimentally validated receptor-bound template. The lack of a published GHRHR cryo-EM or crystal structure with a bound agonist further limits template-based reasoning.

Despite the failed structure prediction, the aggregation propensity (0.155) and stability score (0.423) of the modified peptide are noteworthy. The low aggregation propensity suggests that D-Ala2 does not introduce significant self-assembly liability, which is relevant for subcutaneous formulation. The moderate stability score and predicted half-life of 1–6 hours (moderate-to-long by peptide standards) are consistent with the hypothesis that reducing N-terminal proteolytic susceptibility confers measurable stability gains even without other backbone modifications. BBB penetration is predicted at 0.053 — essentially zero — which is expected and appropriate for a large, hydrophilic peptide acting on pituitary receptors accessible from systemic circulation.

The literature landscape for this modification is characterized by strong mechanistic plausibility but notable direct evidence gaps. The (3-29) metabolite detection is the strongest available indirect evidence for DPP-IV activity on sermorelin in vivo, but no

published study has directly quantified DPP-IV's relative contribution to sermorelin clearance versus other proteases (NEP, aminopeptidases, endopeptidases). This is a critical uncertainty: if DPP-IV accounts for only 20–30% of clearance, a DPP-IV-blocking modification may extend half-life modestly rather than dramatically. The GHRHR agonist SAR at position 2 is also uncharacterized for stereochemistry — while D-Ala preserves the methyl side chain, the backbone geometry change could theoretically alter helix-dipole interactions or receptor N-terminal capping contacts.

From a performance and longevity research perspective, the potential impact of this modification is significant. If validated, D-Ala2-Sermorelin would offer a simpler, single-modification analog with extended dosing intervals compared to native Sermorelin, potentially improving the pulsatile GH secretion profile and reducing injection burden. This positions it conceptually between native Sermorelin and the more extensively modified CJC-1295, occupying an untested but pharmacologically plausible middle ground. The absence of a DAC (drug affinity complex) conjugate means receptor downregulation risk may be lower than with ultra-long-acting analogs.

The honest assessment of this FOLD is that the computational infrastructure has reached its informational ceiling for this peptide. The structural prediction failed to resolve the key question of whether D-Ala2 preserves the GHRHR-binding helical conformation. The modification hypothesis is well-supported by chemical logic and pharmacological precedent, but the in silico evidence is insufficient to assign confidence to the predicted binding outcome. This DISTILLATION is best understood as a formally documented, mechanistically motivated hypothesis awaiting wet-lab entry — a crystallized research direction rather than a validated finding.

RESEARCH BRIEF

MECHANISM OF ACTION

Sermorelin acts as a full agonist at the growth hormone-releasing hormone receptor (GHRHR), a class B G protein-coupled receptor (GPCR) expressed primarily on somatotroph cells of the anterior pituitary gland. Upon binding, it activates Gαs, elevating intracellular cAMP, activating PKA, and triggering phosphorylation of CREB — the canonical signaling cascade driving GH gene transcription and pulsatile GH secretion. The N-terminal region of GHRH/Sermorelin (residues 1–9, particularly Tyr1, Asp3, Ile5, Phe6) forms the primary pharmacophore responsible for receptor activation, engaging the receptor's transmembrane bundle and juxtamembrane extracellular domain. The C-terminal helical region (residues 15–29) contributes to binding affinity through interactions with the receptor's extracellular domain but is not required for efficacy per se.

In native Sermorelin, the Tyr1-Ala2 N-terminal dipeptide is recognized by dipeptidyl peptidase-IV (DPP-IV, CD26), a ubiquitous serine exopeptidase present in plasma, endothelial surfaces, and lymphocytes. DPP-IV cleaves the Tyr1↓Ala2 bond (releasing the Tyr1-Ala2 dipeptide), generating sermorelin(3-29) — a truncated, biologically inactive fragment that cannot engage GHRHR with meaningful affinity. This cleavage is the dominant early inactivation event for native Sermorelin, with a plasma half-life of approximately 7 minutes.

The D-Ala2 substitution in this FOLD blocks DPP-IV recognition through stereochemical incompatibility. DPP-IV's S1 subsite is stereospecific for L-configured amino acids at the P1 position; a D-Ala at position 2 cannot be accommodated in the enzyme's active site geometry, preventing catalytic cleavage. The side chain (methyl group) is chemically identical, so no receptor pharmacophore contact mediated by the side chain is expected to be disrupted. The backbone geometry perturbation is localized to the α-carbon chirality and may influence local helix-dipole orientation at the N-cap — a structural detail that cannot be resolved without an experimental structure.

PERFORMANCE APPLICATIONS

Sermorelin and its analogs occupy a well-defined niche in the performance and longevity research space as GHRH-axis secretagogues. Unlike direct GH or IGF-1 administration, Sermorelin stimulates endogenous, pulsatile GH release — preserving hypothalamic-pituitary axis feedback, avoiding GH receptor desensitization, and maintaining physiologically appropriate GH pulse amplitude and frequency. This mechanism is considered by researchers to offer a more biomimetic approach to GH axis support.

Tissue remodeling and body composition: GH and downstream IGF-1 promote lipolysis in adipose tissue, nitrogen retention, and skeletal muscle protein synthesis. GHRH secretagogues have documented effects on lean mass preservation and visceral fat reduction (tesamorelin's FDA approval for HIV-associated lipodystrophy is the clearest clinical example).

Recovery and regenerative biology: GH is a key mediator of collagen synthesis, extracellular matrix remodeling, and satellite cell activation in skeletal muscle. Optimized GHRH-axis stimulation is a recognized research focus in injury recovery, tendon/ligament biology, and post-surgical rehabilitation contexts.

Sleep architecture: Endogenous GH is secreted predominantly during slow-wave sleep. GHRH agonists have been investigated for their ability to deepen and consolidate slow-wave sleep cycles — a potential performance and cognitive recovery application independent of direct anabolic effects.

Longevity and somatopause: The progressive decline in GH pulse amplitude with aging (somatopause) is associated with body composition changes, reduced bone

mineral density, and metabolic dysfunction. GHRH-axis restoration is an active area of anti-aging research.

A D-Ala2-Sermorelin with extended half-life would reduce dosing frequency (potentially from daily to every-other-day subcutaneous injection), improve pharmacokinetic consistency, and may provide more sustained GH pulse augmentation — a meaningful practical improvement for research use cases requiring chronic administration.

MODIFICATION RATIONALE

The D-Ala2 substitution is a single-atom stereocentre inversion with a precise mechanistic target: abolition of DPP-IV N-terminal cleavage. The chemical logic is as follows:

DPP-IV substrate profile: DPP-IV (EC 3.4.14.5) is a prolyl oligopeptidase family serine protease that cleaves Xaa-Pro↓ and Xaa-Ala↓ bonds from peptide N-termini when position 2 carries a Pro or Ala (or similar small residues) in the L-configuration. Sermorelin's Tyr1-Ala2 sequence matches this profile perfectly. The S1 subsite of DPP-IV is a deep, stereospecific pocket that cannot accommodate D-configured P1 residues due to steric and geometric incompatibility with the catalytic machinery.

Precedent in GHRH analogs: The field has independently arrived at N-terminal modifications as the primary strategy for extending GHRH analog half-life. CJC-1295 uses a combination of modifications including Ala8→Aib and reactive maleimide conjugation. Tesamorelin replaces the free N-terminus with a trans-3-hexenoic acid conjugate. The D-Ala2 approach is simpler than both — a single stereoinversion requiring no exotic chemistry — and directly targets the identified DPP-IV cleavage site.

Evidence for Tyr1-Ala2 as the primary cleavage site: The detection of sermorelin(3-29)-NH2 as a distinct urinary metabolite in anti-doping mass spectrometry studies (PMID:37806509) provides direct metabolomics evidence that this bond is cleaved in vivo. The differential stability of N-terminal vs. C-terminal sermorelin fragments in serum (PMID:37688464) corroborates N-terminal vulnerability. These data points, while from analytical chemistry contexts rather than mechanistic pharmacology, converge on the Tyr1-Ala2 bond as the primary site of biological instability.

Receptor tolerance of D-Ala at position 2: Position 2 is not a primary GHRHR pharmacophore contact. The key N-terminal activation residues are Tyr1, Asp3, Ile5, and Phe6. Position 2 occupies the peptide backbone connecting Tyr1 to the active pharmacophore, and the Ala2 side chain does not make specific receptor contacts per homology inference from other class B GPCR structures. D-Ala preserves the methyl side chain while altering only backbone chirality — a conservative

modification from a receptor pharmacology perspective, though stereospecificity of the GHRHR binding groove at this position has not been experimentally established.

STABILITY ANALYSIS

All values are computational predictions. No experimental validation has been performed.

Parameter	Wild-type Sermorelin	D-Ala2-Sermorelin (predicted)
DPP-IV susceptibility	High (Tyr-Ala N-terminal motif)	Predicted abolished
Plasma half-life	~7 min (literature)	~1–6 hours (in silico estimate)
Aggregation propensity	Not determined	0.155 (low)
Stability score	Not determined	0.423 (moderate)
BBB penetration	Negligible (expected)	0.053 (negligible, predicted)
pLDDT (structural confidence)	—	0.494 (low — structure DISCARDED)

DPP-IV resistance: The stereochemical rationale for complete DPP-IV resistance is well-founded. If DPP-IV is the dominant degradation pathway, the predicted half-life extension to the 1–6 hour range (versus ~7 minutes for native Sermorelin) would represent a 10–50-fold improvement — comparable to early-generation D-amino acid substituted GLP-1 analogs before PEGylation and albumin-binding strategies were introduced.

Caveat on other proteases: DPP-IV is unlikely to be the only proteolytic pathway. Neutral endopeptidase (NEP/neprilysin), non-specific aminopeptidases, and plasma endopeptidases likely contribute to overall clearance. D-Ala2 blocks only one pathway; the residual clearance by other enzymes will determine the actual half-life ceiling. This limits the maximum achievable half-life extension and prevents direct comparison to CJC-1295 (which uses multi-site modifications and albumin-binding conjugation).

Aggregation propensity (0.155): This low value suggests the modification does not introduce hydrophobic aggregation liability, which is practically relevant for subcutaneous formulation stability and injection-site tolerability.

BBB penetration (0.053): As expected for a 29-residue hydrophilic peptide, CNS access is negligible. GHRHR is expressed on pituitary somatotrophs accessible via systemic circulation, so this is not a therapeutic limitation.

Structural confidence: The pLDDT of 0.494 means the predicted atomic coordinates are unreliable and should not be used for binding geometry analysis. The low confidence reflects the intrinsic disorder of Sermorelin in free solution — GHRH-family peptides adopt helical structure principally upon receptor engagement. This is a limitation of single-sequence structure prediction tools rather than evidence of a destabilized or misfolded peptide.

RESEARCH DIRECTIONS

This FOLD defines a hypothesis with strong mechanistic support and a clear experimental path to validation. The following research program would systematically test the key claims:

Priority 1 — DPP-IV resistance assay (in vitro): Incubate synthetic D-Ala2-Sermorelin with purified recombinant DPP-IV (or plasma) and compare N-terminal cleavage kinetics versus native Sermorelin by LC-MS/MS monitoring of the (3-29) fragment. This is a straightforward, low-cost experiment that directly tests the primary hypothesis. Expected result: complete abolition of (3-29) fragment generation with D-Ala2 modification.

Priority 2 — Plasma stability half-life determination: Incubate both peptides in fresh human plasma at 37°C and measure intact peptide remaining over time by HPLC or MS. This provides an integrated measure of all proteolytic pathways, directly answering whether DPP-IV is the rate-limiting step in overall clearance.

Priority 3 — GHRHR binding and functional assay: (a) Competitive radioligand binding assay with [125I]-GHRH to measure binding affinity (Ki) of D-Ala2-Sermorelin versus native Sermorelin at GHRHR-expressing cells (e.g., rat pituitary cells or HEK293 cells stably expressing GHRHR). (b) cAMP accumulation assay to measure agonist potency (EC50) and maximum efficacy (Emax). These two assays together establish whether D-Ala2 preserves both binding and functional activation.

Priority 4 — In vivo GH secretion model: In rodent models (rat or mouse), compare the GH secretion time-course following equivalent subcutaneous doses of native Sermorelin versus D-Ala2-Sermorelin. Key endpoints: peak GH, area under the GH-time curve, and duration of GH elevation. This translates in vitro findings to physiologically relevant pharmacodynamics.

Priority 5 — Structural characterization: CD spectroscopy to confirm helical content in membrane-mimetic environments (TFE or DPC micelles). If resources permit, cryo-EM of D-Ala2-Sermorelin bound to GHRHR would resolve the binding geometry question definitively and provide a template for future FOLD runs.

Future FOLD directions: If D-Ala2 is confirmed to preserve activity, subsequent DISTILLATIONs could explore additive modifications — D-Ala2 combined with a C-terminal amide, or D-Ala2 combined with an Aib substitution at another protease-

susceptible position — to approach CJC-1295-level stability with a simpler chemical scaffold.

SEQUENCES

NATIVE

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YADAIFTNSYRKVLGQLSARKLLQDIMSR
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MODIFIED

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Y-(D-Ala)-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
- structural prediction was DISCARDED (pLDDT 0.494) — no reliable 3D binding geometry could be predicted; all structural inferences in this report are based on mechanistic reasoning, not computational structural data
- DPP-IV has not been directly confirmed as the dominant degradation pathway for sermorelin — other proteases (NEP, aminopeptidases, endopeptidases) may account for a substantial fraction of clearance, limiting the magnitude of half-life extension achievable by this single modification
- GHRHR stereospecificity at position 2 has not been experimentally characterized — D-Ala2 could reduce receptor binding affinity or agonist efficacy despite preserving the side-chain identity, and no published SAR data for this position in sermorelin exists
- the predicted half-life estimate (1–6 hours) is a computational inference based on general stability scoring, not a pharmacokinetic model validated against experimental sermorelin data
- D-Ala2-Sermorelin has not been described in the peer-reviewed literature — this is a novel, untested molecular hypothesis
- the (3-29) metabolite evidence cited is from anti-doping analytical chemistry, not mechanistic pharmacology; it is hypothesis-generating, not confirmatory

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) — — Cationic exchange SPE combined with triple quadrupole UHPLC-MS/MS for detection of GHRHs in urine samples
4. **PMID —** (2023) — — In-house standards derived from doping peptides: Enzymatic and serum stability and degradation profile of GHRP and GHRH-related peptides
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 —** (2025) — — Growth Hormone-Releasing Hormone Antagonists Increase Radiosensitivity in Non-Small Cell Lung Cancer Cells
8. **PMID —** (2019) — — Growth Hormone-Releasing Hormone Receptor Antagonist Modulates Lung Inflammation and Fibrosis due to Bleomycin
9. **PMID —** (2021) — — A potentially effective drug for patients with recurrent glioma: sermorelin