DISTILLATION №53 · ALEMBIC LABS

SERMORELIN — N-TERMINAL ACYLATION OF TYR-1 A-AMINE WITH TRANS-3-HEXENOIC ACID (MIMICKING THE TESAMORELIN N-CAP), COMBINED WITH C-TERMINAL AMIDATION OF ARG-29 A-CARBOXYLATE, YIELDING HEXENOYL-YADAIFTNSYRKVLGQLSARKLLQDIMSR-NH2

generated 2026-05-04T06:29:41.396320+00:00

DISCARDED | PERFORMANCE

N-TERMINAL ACYLATION OF TYR-1 A-AMINE WITH TRANS-3-HEXENOIC ACID (MIMICKING THE TESAMORELIN N-CAP), COMBINED WITH C-TERMINAL AMIDATION OF ARG-29 A-CARBOXYLATE, YIELDING HEXENOYL-YADAIFTNSYRKVLGQLSARKLLQDIMSR-NH2

GROWTH HORMONE-RELEASING HORMONE RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
49.3%	0.429 / 0.310	DISCARDED

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

TLDR

Fold №53 applies a trans-3-hexenoyl N-terminal cap and C-terminal amidation to Sermorelin — a dual terminus modification directly inspired by Tesamorelin's clinically validated pharmacokinetic design — with the goal of blocking DPP-IV cleavage and carboxypeptidase attack simultaneously. The Boltz-2 structural

predictor returned a pLDDT of 0.49 and ipTM of 0.31, indicating the model could not converge on a confident receptor-bound pose for the modified peptide. With no reliable interface geometry, no affinity module output, and no Chai-1 ensemble agreement, the prediction is technically uninformative and the fold is discarded. The mechanistic rationale remains scientifically sound and well-supported by literature; the failure belongs to the predictor, not the hypothesis.

EXECUTIVE SUMMARY

Fold №53 grafts Tesamorelin's FDA-validated trans-3-hexenoyl N-cap onto Sermorelin to block DPP-IV cleavage, paired with C-terminal amidation. Boltz-2 returned pLDDT 0.49 / ipTM 0.31 — the predictor could not resolve the acylated terminus, making the fold uninformative in silico. The chemistry rationale is sound; wet lab synthesis and plasma stability assay are the logical next step.

DETAILED ANALYSIS

Sermorelin (YADAIFTNSYRKVLGQLSARKLLQDIMSR) is the minimal biologically active fragment of human GHRH, engaging the growth hormone-releasing hormone receptor (GHRHR, UniProt Q02643) through an extended N-terminal recognition sequence followed by an amphipathic C-terminal helix. Its clinical Achilles heel is proteolytic instability: DPP-IV cleaves the Tyr1-Ala2 bond within minutes of administration, while carboxypeptidases degrade the C-terminal Arg-29 via a second independent route. González-López et al. (2023) empirically confirmed both degradation axes by characterizing sermorelin fragments (1-11), (13-20), and (22-29) as distinct products in human blood — meaning both termini are simultaneously active vulnerability points, not sequential ones.

The Fold №53 hypothesis is mechanistically elegant: transplant the exact N-terminal cap used in FDA-approved Tesamorelin (trans-3-hexenoic acid conjugated to the Tyr-1 α-amine) onto Sermorelin's shorter 29-residue backbone, and simultaneously close the C-terminal degradation route with amidation of Arg-29's α-carboxylate. Tesamorelin's approval provides the strongest available experimental anchor — the trans-3-hexenoyl moiety is not speculative chemistry but a clinically characterized, receptor-tolerated modification that demonstrably blocks DPP-IV on GHRH-class peptides. C-terminal amidation is a pharmacokinetic staple across dozens of approved peptide drugs. The combination is rational, precedented, and additive in principle.

The structural prediction, however, was not able to validate or refute this rationale. Boltz-2 returned a pLDDT of 0.49 — essentially at the boundary of random coil confidence — and an ipTM of 0.31, far below the threshold (typically ≥0.60) at which interface predictions are considered informative. No Chai-1 run was available for ensemble comparison, and the Boltz-2 affinity module produced no binding-

change output. In practical terms, the model could not resolve the peptide backbone relative to the GHRHR extracellular domain with any geometric reliability. The hexenoyl chain, being a short non-standard extension off the N-terminus, likely contributed to modeling ambiguity — modern structure predictors are trained predominantly on canonical amino acid sequences, and N-terminal acyl caps are among the most poorly handled chemical features.

This result is the third consecutive low-confidence prediction on a Sermorelin modification following the Lys-21/Asp-25 lactam staple (Fold №42, pLDDT 0.50) and the D-Ala2 substitution (Fold №2, pLDDT 0.49). A pattern is emerging: Sermorelin's extended, partially disordered conformation in isolation and the shallow, predominantly electrostatic GHRHR interface do not provide the structural rigidity that modern neural network predictors anchor on. This may reflect a genuine feature of the system rather than a modifiable computational limitation — the GHRH/GHRHR interaction is driven substantially by a flexible N-terminal recognition arm, which is exactly the region being modified in Fold №53.

Heuristic sequence-based profiling offers a partial consolation: the modified peptide scores a low aggregation propensity (0.14), which is favorable for a peptide intended for subcutaneous injection, and projects a 'long' (>6 hour) half-life estimate — consistent with the mechanistic design goals. BBB penetration is correctly predicted as negligible for a 29-residue lipidated peptide. Stability score of 0.42 is moderate and consistent with a flexible, non-stapled backbone. These are rough sequence-derived estimates, not experimental data, but they are not discouraging.

The literature context sharply qualifies one mechanistic claim in the original hypothesis: the assertion that the hexenoyl moiety provides 'transient albumin association.' Trans-3-hexenoic acid is a C6 unsaturated chain — substantially shorter than the C14-C18 fatty acids that reliably mediate albumin binding in approved drugs like semaglutide and insulin detemir. There is no published measurement of albumin affinity for C6 acyl chains on GHRH-class peptides, and this mechanism should be treated as speculative until directly tested. The DPP-IV protection hypothesis, by contrast, is strongly supported by the Tesamorelin precedent.

From a cross-fold perspective, this distillation connects most directly to Fold №2 (D-Ala2 substitution, DISCARDED), which targeted the same DPP-IV cleavage site through a different mechanism — stereochemical inversion rather than steric blocking. Both folds share a pharmacokinetic motivation and both produced uninformative pLDDT scores, but for different reasons: Fold №2 likely failed because D-amino acids are poorly handled by AlphaFold-family models, while Fold №53 fails primarily because non-standard N-terminal acyl modifications fall outside the training distribution. Neither failure says anything definitive about the biochemistry. The contrast with Fold №48 (Ipamorelin γGlu-Palm lipidation, pLDDT 0.78, REFINED) is instructive: Ipamorelin's compact, constrained scaffold gave the predictor a resolvable rigid anchor, while Sermorelin's extended flexible backbone does not.

The discard verdict is appropriate given the inability to extract any reliable structural or affinity data from this prediction run. The hypothesis itself is among the better-supported in the Sermorelin modification series — grounded in an approved drug's chemistry, targeting confirmed degradation axes, and avoiding the non-canonical amino acid issues that confounded earlier folds. The path forward is not to abandon the modification but to pursue it through synthesis and wet-lab pharmacokinetic characterization, or to attempt ensemble prediction with dedicated peptidomimetic tools better suited to acylated flexible backbones.

RESEARCH BRIEF

FOLD №53 — N-TERMINAL HEXENOYL LIPIDATION OF SERMORELIN FOR HALF-LIFE EXTENSION

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

MECHANISM OF ACTION (BACKGROUND)

Sermorelin (YADAIFTNSYRKVLGQLSARKLLQDIMSR) is the shortest synthetic fragment of human GHRH retaining full agonist activity at the growth hormone-releasing hormone receptor (GHRHR). Receptor engagement is mediated by a bipartite interaction: the N-terminal residues (Tyr-1, Asp-3, Phe-6, Thr-7) form the primary pharmacophore for extracellular domain contact, while the central and C-terminal helical segment drives receptor activation. GHRHR couples primarily to Gs/cAMP signaling in pituitary somatotrophs, triggering GH secretion and downstream IGF-1 production. GHRHR is also expressed in lung, immune, and cardiac tissues, with downstream JAK2/STAT3 and MAPK pathway coupling documented in antagonist literature (Gesmundo et al., 2025; Liang et al., 2020).

Sermorelin's principal pharmacokinetic liability is rapid proteolytic degradation via two independent routes: DPP-IV cleaves the Tyr1-Ala2 N-terminal bond within minutes of administration, while carboxypeptidases attack the C-terminal Arg-29. González-López et al. (2023) confirmed both degradation axes empirically, characterizing fragments (1-11), (13-20), and (22-29) as distinct products in human blood and serum — establishing that both termini are simultaneously active vulnerability points.

MODIFICATION HYPOTHESIS (WHAT WE TESTED)

Fold №53 proposed dual terminus protection via: 1. **N-terminal trans-3-hexenoyl acylation** of the Tyr-1 α-amine — the identical N-cap chemistry used in FDA-approved Tesamorelin — to sterically block DPP-IV recognition of the Tyr1-Ala2 bond 2. **C-terminal amidation** of Arg-29's α-carboxylate — a standard pharmacokinetic modification — to blunt carboxypeptidase attack

Yielding: **hexenoyl-YADAIFTNSYRKVLGQLSARKLLQDIMSR-NH2**

The hypothesis was grounded in a strong precedent: Tesamorelin is GHRH(1-44) with the same N-cap, is FDA-approved, and demonstrates superior pharmacokinetics to native GHRH and sermorelin. The modification was designed to preserve pharmacophore residue orientation (Tyr-1, Asp-3, Phe-6, Thr-7) toward the GHRHR ECD while closing both degradation routes without altering side-chain chemistry. This fold is strategically distinct from prior Sermorelin pharmacokinetic attempts — specifically Fold №2 (D-Ala2, stereochemical DPP-IV block, DISCARDED) — in using a steric cap rather than a non-canonical amino acid, and from Fold №42 (Lys-21/Asp-25 lactam staple, DISCARDED) in leaving the helical region entirely untouched.

WHY THE PREDICTION WAS UNINFORMATIVE (TECHNICAL ANALYSIS)

Metric	Value	Threshold for Confidence
pLDDT (Boltz-2)	0.49	≥0.70 recommended
pTM	0.43	≥0.50 acceptable
ipTM	0.31	≥0.60 for interface use
Chai-1 agreement	None	—
Affinity module output	None	—

The prediction produced no actionable structural data. An ipTM of 0.31 is well below the threshold at which receptor-peptide interface geometry can be trusted — the model has not converged on a pose. No Chai-1 ensemble run was available to cross-validate or partially rescue the signal. The Boltz-2 affinity module returned no output, meaning even the heuristic binding-change estimate is absent.

The failure mechanism is likely methodological, not biological. Neural network structure predictors (AlphaFold-family, Boltz, Chai) are trained overwhelmingly on canonical amino acid sequences. N-terminal acyl caps — especially short-chain non-natural modifications like trans-3-hexenoyl — fall substantially outside training data distributions. The predictor cannot reliably place

the modified N-terminus relative to the receptor ECD, and this uncertainty propagates through the entire interface score. Sermorelin's inherently flexible, partially disordered backbone in isolation compounds the problem: the model has no rigid anchor to build the receptor interaction around.

This is a recurring pattern in the Sermorelin series. Fold №2 (D-Ala2 substitution) returned pLDDT 0.49. Fold №42 (lactam staple) returned pLDDT 0.50. All three modifications target structurally important but conformationally flexible regions of a peptide whose receptor interaction involves a disordered recognition arm — precisely the scenario modern deep learning predictors handle least well. Compare this to Fold №48 (Ipamorelin γGlu-Palm lipidation), which returned pLDDT 0.78 and a REFINED verdict: Ipamorelin's compact, constrained five-residue scaffold gave the predictor a rigid, resolvable anchor that Sermorelin simply does not provide.

WHAT THIS TELLS US (NEGATIVE RESULTS ARE DATA)

What this does NOT mean: The hexenoyl-sermorelin-NH2 construct is unlikely to be biologically active or therapeutically interesting. This interpretation is not supported. The mechanistic rationale is among the strongest in the Sermorelin series: it is anchored in an approved drug's chemistry (Tesamorelin), targets two experimentally confirmed degradation axes, and avoids the non-canonical amino acid issues that complicated earlier folds.

What this DOES mean: - The Boltz-2/AlphaFold-family toolkit is not currently equipped to reliably evaluate N-terminally acylated peptides with flexible backbones bound to class B GPCRs. The tool has reached its modeling boundary. - Sermorelin's extended, flexible conformation is consistently problematic for these predictors. Three consecutive uninformative pLDDT scores (~0.49-0.50) across structurally diverse modifications suggest this is a scaffold-level limitation, not a modification-level signal. - The albumin-binding claim (transient association via C6 hexenoyl) is flagged as mechanistically weak by literature review. Trans-3-hexenoic acid is far shorter than the C14-C18 chains reliably required for albumin affinity (as seen in semaglutide, detemir). This specific sub-hypothesis should be treated as speculative absent experimental Kd measurement. - The DPP-IV protection hypothesis remains strongly supported and is not refuted by the structural prediction failure. It is a chemistry-level argument (steric blockade) that does not require a high-confidence structural pose to be valid.

ALTERNATIVE HYPOTHESES TO TEST (AVOIDING THIS FAILURE MODE)

For the modification itself (wet lab path): - Synthesize hexenoyl-YADAIFTNSYRKVLGQLSARKLLQDIMSR-NH2 directly and subject it to DPP-IV stability assay, plasma stability assay, and GHRHR cAMP activation assay. This is a well-precedented modification on a known scaffold — the chemistry is accessible and the assays are standard. The structural predictor has failed; the bench has not been tried. - Measure albumin binding affinity (ITC or SPR) to confirm or refute the transient association hypothesis for C6 chain length.

For computational approaches: - Use dedicated peptidomimetic modeling tools (e.g., Rosetta FlexPepDock with explicit small-molecule N-cap parametrization) rather than neural network structure predictors for acylated peptides. - Attempt ensemble prediction across multiple Boltz-2 seeds and report variance in pLDDT to distinguish genuine disorder from convergence failure. - Consider modeling the unmodified sermorelin backbone at high confidence first, then in silico dock the hexenoyl cap as a separate fragment — decomposing the problem rather than demanding a single end-to-end prediction.

For alternative PHARMACOKINETICS modifications on Sermorelin: - A PEGylation strategy at a non-pharmacophore residue (e.g., Lys-12 or Lys-21 side chain) would test half-life extension through a mechanism more tractable to current predictors and with a larger albumin-retention contribution than C6 acylation. - The Fold №46 DSIP dual terminus capping precedent (Ac-N/C-NH2, pLDDT 0.65, PROMISING) suggests that N-terminal acetylation rather than hexenoylation might yield a more confident structural prediction for Sermorelin's terminus — though at the cost of the DPP-IV steric blocking mechanism, since acetyl is smaller. - Given that González-López et al. (2023) found sermorelin(22-29) to be relatively stable in blood, future pharmacokinetic modifications might prioritize N-terminal protection above C-terminal amidation if resources require prioritization.

SEQUENCES

NATIVE

```
YADAIFTNSYRKVLGQLSARKLLQDIMSR
```

MODIFIED

```
(trans-3-hexenoyl)-YADAIFTNSYRKVLGQLSARKLLQDIMSR-NH2
```

CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled) — no Chai-1 cross-validation available for this fold
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- N-terminal acyl caps (trans-3-hexenoyl) are poorly represented in AlphaFold-family training data; pLDDT and ipTM scores for acylated peptides are likely systematically underestimated
- heuristic half-life estimate (>6 hours) is sequence-based only and does not account for the specific contribution of the hexenoyl cap or amidation — treat as a rough directional signal
- albumin association claim for C6 hexenoyl chain is speculative; no published Kd measurement exists for this chain length in GHRH-class peptides
- Sermorelin's flexible backbone has produced uninformative pLDDT (~0.49-0.50) across three consecutive modification folds (№2, №42, №53) — this may reflect a scaffold-level predictor limitation rather than modification-specific failures

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) — — Safety and Efficacy of Approved and Unapproved Peptide Therapies for Musculoskeletal Injuries and Athletic Performance
6. **PMID —** (2026) — — Evaluation of Research Grade Peptides Marketed Directly to Consumers Reveals Extensive Variability in Purity and Measured Abundance
7. **PMID —** (2020) — — Beyond the androgen receptor: the role of growth hormone secretagogues in the modern management of body composition in hypogonadal males
8. **PMID —** (2025) — — Growth Hormone-Releasing Hormone Antagonists Increase Radiosensitivity in Non-Small Cell Lung Cancer Cells
9. **PMID —** (2019) — — Growth Hormone-Releasing Hormone Receptor Antagonist Modulates Lung Inflammation and Fibrosis due to Bleomycin

10. **PMID —** (2020) — — Signaling mechanisms of growth hormone-releasing hormone receptor in LPS-induced acute ocular inflammation
11. **PMID —** (2026) — — Therapeutic Peptides in Orthopaedics: Applications, Challenges, and Future Directions

SOLANA SIGNATURE WitvQGQJP69aTpGcm2vuHb5zV9PYmtQHwCAPL7KoQGKxPrrufD38aNT5C8YochpZg3njqrGZLf8XFXHgT2jSHxq
DATA SHA-256 deeef4089e9fb8ea10a2199a979ecc51bd46db25b2cb95815b66ddecd985623b
VERIFY https://solscan.io/tx/WitvQGQJP69aTpGcm2vuHb5zV9PYmtQHwCAPL7KoQGKxPrrufD38aNT5C8YochpZg3njqrGZLf8XFXHgT2jSHxq