DISTILLATION №21 · ALEMBIC LABS

EPITALON — C-TERMINAL AMIDATION OF GLY-4 (FREE -COOH → -CONH2), YIELDING AEDG-NH2

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DISCARDED | LONGEVITY | C-TERMINAL AMIDATION OF GLY-4 (FREE -COOH → -CONH2), YIELDING AEDG-NH2 | TELOMERASE REVERSE TRANSCRIPTASE

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
34.2%	0.203 / 0.208	DISCARDED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Telomerase reverse transcriptase	O14746	—

TLDR

Fold #21 tests C-terminal amidation of Epitalon (AEDG → AEDG-NH2) as a carboxypeptidase-resistant analog, following the pLDDT collapse of the D-Ala N-terminal variant in Fold #6. The structural prediction returned an identical pLDDT of 0.34 and an ipTM of 0.21, confirming that a 4-residue peptide is simply too short and too disordered for AlphaFold-class tools to resolve a confident complex geometry. The amidation modification is pharmacologically rational, but the prediction infrastructure cannot evaluate it meaningfully at this sequence length. The DISCARDED verdict reflects a tool-resolution failure rather than evidence against the modification's biological merit.

EXECUTIVE SUMMARY

Epitalon AEDG-NH₂: pLDDT 0.34, ipTM 0.21 — identical to Fold #6's D-Ala collapse. Two terminus-only strategies, same prediction floor. The 4-mer is simply too short for AF2-class tools; plasma stability assay and hTERT reporter are the right next moves.

DETAILED ANALYSIS

Epitalon (Ala-Glu-Asp-Gly, AEDG) is a synthetic tetrapeptide derived from the bovine pineal peptide complex Epithalamin. Over roughly 25 years of published research, it has accumulated evidence for telomerase upregulation — specifically hTERT mRNA induction and enzymatic activation — alongside broader geroprotective, antioxidant, and cytoprotective effects across rodent, bovine, and human cell systems. The most mechanistically detailed proposal in the literature (PMID:32019204) places AEDG's action upstream of hTERT at the level of histone H1 binding, implying an epigenetic transcriptional route rather than direct enzymatic engagement of the telomerase complex. This mechanistic ambiguity is important context for evaluating any chemical modification.

The modification under investigation in this fold is C-terminal amidation: conversion of the Gly-4 free carboxylate (-COOH) to an amide (-CONH2), yielding AEDG-NH2. The chemical rationale is well-grounded. Carboxypeptidase A and B family enzymes recognize and cleave C-terminal free acids; replacing the carboxylate with a neutral amide removes the electrostatic recognition element for these exoproteases. C-terminal amidation is a validated medicinal chemistry strategy employed across many short peptide therapeutics and is isosteric with the carboxylate at the level of heavy atom count and volume, meaning steric penalties are minimal. The modification also adds a hydrogen-bond donor (the amide NH2) that could conceivably enhance interactions with carbonyl-accepting residues on target proteins such as the histone H1 basic domain.

The hypothesis was developed directly in response to lessons from Fold #6, which tested N-terminal D-Ala substitution on the same peptide and returned a pLDDT of 0.34 — a complete structural collapse. The team correctly inferred that backbone chirality changes are poorly tolerated for this marginal compact fold and pivoted to a backbone-preserving, terminus-only modification targeting the opposite exoprotease pathway. This is methodologically sound reasoning: exhausting N-terminal and C-terminal protection strategies in sequence allows the lab to map the degradation vulnerability landscape of AEDG systematically.

Unfortunately, the structural prediction for AEDG-NH2 returned nearly identical metrics to Fold #6: pLDDT 0.34, pTM 0.20, ipTM 0.21. The predicted complex shows a disordered 4-mer with no convergent fold and a poorly defined interface. Critically, these scores are not meaningfully distinguishable from the D-Ala collapse — the prediction has not failed because the amidation is destabilizing, but because a 4-residue peptide falls below the effective resolution floor of structure prediction tools trained on longer, more structured proteins. The Boltz-2 affinity module returned no values, and no Chai-1 agreement metric was available. The heuristic sequence-based profile (stability score 0.504, short half-life estimate, low BBB penetration) reflects the parent peptide's intrinsic properties more than the amidation delta.

The literature context is relevant but cannot rescue the prediction. The strongest mechanistic evidence for telomerase engagement (PMID:40908429, Al-Dulaimi et al. 2025) was subject to a published correction (PMID:41240216), warranting interpretive caution. The histone H1 binding proposal (PMID:32019204) implies that the Glu-2/Asp-3 acidic face and potentially the Gly-4 carboxylate engage basic Lys/Arg residues on histone H1 — in which case removing the C-terminal negative charge via amidation could actually impair, rather than preserve, target engagement. This is a genuine pharmacological risk that wet-lab SAR studies would need to resolve and that computational tools at this length scale cannot adjudicate.

From a cross-fold perspective, Fold #21 closes a logical pair with Fold #6: both N-terminal and C-terminal protection strategies for Epitalon have now returned pLDDT 0.34, establishing a ceiling on what current structure prediction tools can deliver for this tetrapeptide against TERT. The contrast with successful longevity peptide folds in this lab — SS-31 variants at pLDDT 0.85 (Folds #11, #17) and MOTS-c K13R at pLDDT 0.63 (Fold #19) — underscores that peptide length and intrinsic structuredness are the primary determinants of prediction confidence, not modification quality.

The DISCARDED verdict should be read as a tool limitation verdict, not a negative biological signal. The amidation modification remains pharmacologically rational. Future work should pursue orthogonal validation approaches: molecular dynamics simulation of AEDG-NH2 in explicit solvent, plasma stability assay (carboxypeptidase B incubation with HPLC quantification), and a cell-based hTERT reporter assay comparing AEDG and AEDG-NH2 at matched concentrations. These wet-lab and simulation approaches are not constrained by the 4-mer length floor that limits structure prediction tools.

RESEARCH BRIEF

DISTILLATION №21 — DISCARDED

EPITALON C-TERMINAL AMIDATION (AEDG-NH2)

Verdict: DISCARDED | pLDDT 0.34 | ipTM 0.21 | Peptide: AEDG-NH2 | Target: hTERT (O14746)

MECHANISM OF ACTION (BACKGROUND)

Epitalon (AEDG) is a 4-residue synthetic tetrapeptide with ~25 years of published research spanning telomerase activation, histone binding, antioxidant defense, and geroprotection. Its best-supported mechanistic proposal positions AEDG as a histone

H1 ligand at DNA-interacting basic domains (PMID:32019204), with downstream epigenetic upregulation of hTERT mRNA as the primary route to telomerase activation — rather than direct binding to the TERT catalytic domain. Functional effects include dose-dependent telomere length extension in human epithelial and fibroblast cells (PMID:40908429), improved bovine oocyte maturation linked to nuclear telomerase relocalization (PMID:39788414), and ROS reduction with spindle protection in post-ovulatory aging oocytes (PMID:35413689). The native peptide's plasma half-life has not been formally quantified in the peer-reviewed literature, but reliance on intranasal delivery and millimolar in vitro concentrations indirectly suggests significant metabolic lability.

MODIFICATION HYPOTHESIS (WHAT WE TESTED)

This fold tested **C-terminal amidation** of Gly-4 — conversion of the free carboxylate (-COOH) to a primary amide (-$CONH_2$) — yielding **AEDG-NH_2**. The pharmacological rationale is established: carboxypeptidase A and B family enzymes recognise and cleave C-terminal free acids; amidation removes the electrostatic recognition element and is isosteric with the carboxylate, imposing minimal steric cost. The modification also introduces a hydrogen-bond donor that could enhance interactions with carbonyl-accepting residues at the putative histone H1 binding site.

This fold was designed as the logical complement to **Fold #6**, which tested N-terminal D-Ala substitution and collapsed to pLDDT 0.34 — attributed to poor tolerance of backbone chirality changes in a marginally compact 4-mer. C-terminal amidation preserves all backbone stereochemistry while targeting the opposite exoprotease degradation pathway, representing the minimal-perturbation strategy indicated by Fold #6's failure mode.

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

Metric	Fold #6 (D-Ala)	Fold #21 (AEDG-NH2)	Threshold for confidence
pLDDT	0.34	**0.34**	≥0.60 suggested
pTM	—	0.20	≥0.50 suggested
ipTM	—	**0.21**	≥0.60 for complex
Chai-1 agreement	—	None	—

Metric	Fold #6 (D-Ala)	Fold #21 (AEDG-NH2)	Threshold for confidence
Boltz-2 affinity	—	No values	—

The scores are statistically indistinguishable from Fold #6. The critical insight is that **both failures returned identical pLDDT values (0.34) despite completely different modifications** — one a backbone chirality change, one a terminal charge-state change. This convergence strongly suggests the scores are not reflecting the quality of the modification at all: they reflect the **hard floor imposed by sequence length**. AlphaFold-class tools are trained predominantly on structured proteins and longer peptides; a 4-residue, intrinsically disordered sequence provides insufficient information for confident fold prediction regardless of terminal chemistry.

Additionally, ipTM of 0.21 means the predicted interface geometry is essentially random — no specific contact pattern with the TERT surface can be extracted. The Boltz-2 affinity module requires a sufficiently confident structural template to operate and returned no values. The heuristic sequence-based profile (stability 0.504, half-life 15–45 min, aggregation propensity 0.0) reflects properties of the amino acid composition and is not sensitive to the terminal amide change.

The prediction failed to evaluate the modification — it failed to model the peptide.

WHAT THIS TELLS US (NEGATIVE RESULTS ARE DATA)

Fold #21, combined with Fold #6, now establishes a clear empirical boundary: **current AF2-class structure prediction tools operating against TERT cannot return actionable metrics for 4-residue Epitalon analogs, regardless of modification type.** This is a meaningful negative result for the lab's computational pipeline rather than for the chemistry.

What this does not tell us: - Whether AEDG-NH_2 retains hTERT-upregulating activity - Whether C-terminal amidation improves plasma stability of AEDG - Whether the Gly-4 carboxylate is a pharmacophore element required for histone H1 engagement

What this does tell us: - The Epitalon scaffold sits below the resolution floor for structure prediction confidence at this target - N-terminal and C-terminal terminus-only protection strategies have been identified as the next logical wet-lab experiments, not further computational folds of the same 4-mer - The pharmacological risk of removing the C-terminal negative charge (potentially disrupting electrostatic engagement with histone H1's Lys/Arg-rich domain) cannot be evaluated computationally and must be addressed by cell-based SAR - Comparison with SS-31 variants (pLDDT 0.85, Folds #11 and #17) and MOTS-c K13R (pLDDT 0.63, Fold #19) confirms that peptide length and intrinsic structuredness —

not modification quality — are the dominant determinants of prediction confidence in this lab

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ALTERNATIVE HYPOTHESES TO TEST (AVOID THE FAILURE MODE)

Computational alternatives: 1. **Molecular dynamics simulation** — explicit-solvent MD (e.g., GROMACS, AMBER) of AEDG and AEDG-NH_2 in free solution or docked against a histone H1 homology model would capture the conformational ensemble of a disordered tetrapeptide without requiring a confident fold prediction as input 2. **Coarse-grained docking** against histone H1 basic domain — the Khavinson 2020 model (PMID:32019204) provides a putative binding site that could be used for blind docking of AEDG vs. AEDG-NH_2 to assess whether carboxylate removal changes binding geometry 3. **Longer constructs** — if the scientific question is TERT engagement, a fusion of AEDG or AEDG-NH_2 to a structured carrier peptide or scaffold might exceed the pLDDT floor and enable meaningful prediction

Wet-lab alternatives (highest priority): 1. **Plasma stability assay** — incubate AEDG and AEDG-NH_2 with human plasma (or purified carboxypeptidase B) and quantify intact peptide by HPLC/LC-MS at 0, 15, 30, 60, 120 min. This directly tests the amidation hypothesis and doesn't require structural prediction 2. **hTERT reporter assay** — compare AEDG vs. AEDG-NH_2 in a cell-based hTERT-luciferase reporter at matched concentrations (0.01–1 mM range) to determine whether amidation preserves, enhances, or abolishes transcriptional activity 3. **Histone H1 binding assay** — SPR or ITC titration of AEDG vs. AEDG-NH_2 against recombinant histone H1 to directly test whether the C-terminal carboxylate contributes to binding affinity 4. **N+C dual protection** — if both termini are independently modulable, a head-to-head comparison of AEDG-NH_2 (Fold #21), Ac-AEDG (N-terminal acetylation, not yet tested), and Ac-AEDG-NH_2 (dual protection) in a stability assay would establish the dominant degradation pathway empirically

SEQUENCES

NATIVE

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AEDG
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MODIFIED

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AEDG-NH2
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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
- pLDDT 0.34 and ipTM 0.21 fall below meaningful confidence thresholds — no structural or binding conclusions can be drawn from this prediction
- 4-residue peptides are below the effective resolution floor for AF2-class tools; DISCARDED verdict reflects tool failure, not modification failure
- heuristic peptide profile (stability, half-life, BBB) is sequence-composition-based and not sensitive to C-terminal amide vs. carboxylate distinction
- Boltz-2 affinity module returned no values — no predicted binding change is available
- the strongest mechanistic paper for AEDG telomerase activation (PMID:40908429) has a published correction (PMID:41240216); mechanistic claims should be interpreted with caution
- C-terminal amidation removes a potentially pharmacophoric negative charge that may engage histone H1 Lys/Arg residues — biological activity of AEDG-NH_2 is empirically unknown
- cancer cell ALT pathway activation by native AEDG (PMID:40908429) raises unresolved safety considerations for any telomerase-activating analog intended for in vivo use

CITATIONS

1. **PMID —** (2025) — — Overview of Epitalon-Highly Bioactive Pineal Tetrapeptide with Promising Properties
2. **PMID —** (2025) — — Epitalon increases telomere length in human cell lines through telomerase upregulation or ALT activity
3. **PMID —** (2025) — — Epitalon-activated telomerase enhance bovine oocyte maturation rate and post-thawed embryo development
4. **PMID —** (2020) — — AEDG Peptide (Epitalon) Stimulates Gene Expression and Protein Synthesis during Neurogenesis: Possible Epigenetic Mechanism
5. **PMID —** (2022) — — Epitalon protects against post-ovulatory aging-related damage of mouse oocytes
6. **PMID —** (2025) — — The Antioxidant Tetrapeptide Epitalon Enhances Delayed Wound Healing in an in Vitro Model of Diabetic Retinopathy
7. **PMID —** (2002) — — Peptides and Ageing

8. **PMID —** (2002) — — Epitalon influences pineal secretion in stress-exposed rats in the daytime
9. **PMID —** (2025) — — Epitalon increases telomere length in human cell lines through telomerase upregulation or ALT activity (preprint)

SOLANA SIGNATURE 33JfJZegt43xNoQjGjYEETCXMRA6hhJsjJ7hgDrLLF5LLrz83mpuqbmRe7XLfVExSLwFovCQZgv6feCUa2iVUpEg
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