DISTILLATION №17 · ALEMBIC LABS

SS-31 — PHE-4 → 1-NAPHTHYLALANINE (1-NAL) SUBSTITUTION AT THE C-TERMINAL AROMATIC RESIDUE (REGIOISOMER OF THE PREVIOUSLY PROMISING 2-NAL VARIANT)

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PROMISING LONGEVITY
PHE-4 → 1-NAPHTHYLALANINE (1-NAL) SUBSTITUTION AT THE C-TERMINAL AROMATIC RESIDUE (REGIOISOMER OF THE PREVIOUSLY PROMISING 2-NAL VARIANT)
CARDIOLIPIN (MITOCHONDRIAL INNER MEMBRANE PHOSPHOLIPID)

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
85.3%	0.161 / 0.000	PROMISING
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Cardiolipin (mitochondrial inner membrane phospholipid)	—	—

TLDR

DISTILLATION №17 explores the 1-Naphthylalanine regioisomer at the C-terminal aromatic position of SS-31 (elamipretide), directly following Fold #11's 2-Nal variant, to probe whether compact intramolecular aromatic stacking (1-Nal) or extended membrane-insertion geometry (2-Nal) better serves the cardiolipin-binding pharmacophore. AlphaFold-based structural prediction returned a pLDDT of 0.85, matching the 2-Nal fold almost exactly and confirming that the backbone architecture of the alternating cationic-aromatic SS-31 motif is preserved. The 1-Nal side chain is predicted to orient its second fused ring back toward the backbone, positioning it in proximity to Dmt-2 in a geometry consistent with intramolecular aromatic contact — a structurally distinct conformation from the 2-Nal variant. No

cardiolipin complex was modeled, so binding affinity differences between the two regioisomers cannot be assessed computationally at this stage; wet-lab biophysical experiments are required to resolve which geometry confers superior CL-binding potency.

EXECUTIVE SUMMARY

SS-31 Phe-4 → 1-Nal: pLDDT 0.85, matching the 2-Nal regioisomer (Fold #11) exactly. The 1-naphthyl ring is predicted to orient toward the backbone in proximity to Dmt-2, consistent with compact intramolecular aromatic stacking. CL-binding differences between the two regioisomers require wet-lab resolution.

DETAILED ANALYSIS

SS-31 (D-Arg-Dmt-Lys-Phe-NH_2, elamipretide/Bendavia/MTP-131) is a mitochondria-targeted tetrapeptide whose primary mechanism of action is direct, high-affinity binding to cardiolipin (CL) at the inner mitochondrial membrane. The alternating cationic-aromatic architecture — with D-Arg and Lys providing electrostatic attraction to the anionic CL headgroup and Dmt-2 and Phe-4 mediating aromatic insertion into the CL-rich membrane leaflet — is the established pharmacophore. Birk et al. (2013) demonstrated that this SS-31/CL interaction inhibits cytochrome c peroxidase activity, prevents CL peroxidation, stabilizes cristae structure, and enables rapid ATP recovery after ischemia. Chavez et al. further showed that SS-31 engages a network of CL-associated OXPHOS proteins at CL-rich protein-lipid interfaces, suggesting that the geometry and depth of aromatic insertion have functional consequences beyond simple membrane anchoring.

This fold is the second in an explicit regiochemical series on SS-31's C-terminal aromatic residue. Fold #11 established that the 2-Nal substitution is structurally tolerated (pLDDT 0.85), with the linear extension of the 2-naphthyl system predicted to project outward and potentially deepen membrane insertion relative to native Phe-4. DISTILLATION №17 now tests the 1-Nal regioisomer, which has identical molecular formula and logP to 2-Nal but a fundamentally different vector: the second fused ring of 1-Nal orients back toward the peptide backbone rather than extending distally, favoring compact intramolecular aromatic contact with Dmt-2 over maximal membrane insertion depth. This is a well-posed structure-activity relationship question with direct precedent in opioid pharmacology (Dmt-Tic analogs) and GnRH antagonist design, where 1-Nal versus 2-Nal regiochemistry routinely produces measurable differences in receptor engagement and conformational preference.

The structural prediction returned a pLDDT of 0.85 — essentially identical to Fold #11's 2-Nal result — confirming high confidence in the backbone conformation of this compact tetrapeptide. The model positions the 1-naphthyl side chain with its

second ring oriented toward the backbone in proximity to the Dmt-2 aromatic system, consistent with the intramolecular aromatic stacking geometry hypothesized in the experimental design. This is structurally meaningful: a tighter Dmt-2/1-Nal aromatic cluster would increase conformational rigidity of the pharmacophore core, which could translate to improved proteolytic stability and potentially sharper CL-binding selectivity, at the cost of reduced membrane insertion depth relative to the more outward-projecting 2-Nal geometry. The heuristic stability score (0.57) and estimated half-life (moderate, 30 min–2 h) are consistent with a modestly improved metabolic profile relative to native SS-31.

Critically, neither the 1-Nal nor the 2-Nal fold has been modeled against cardiolipin or any CL-protein complex, because cardiolipin is a lipid rather than a structured protein target. This is a fundamental limitation: pLDDT and pTM scores describe peptide backbone confidence, not membrane-binding affinity, insertion depth, or mitochondrial uptake. The pTM of 0.16 reflects the absence of a complex model, not a structural failure. The biological question — which regioisomer binds CL more potently and engages CL-associated OXPHOS proteins more effectively — cannot be resolved computationally with current tools and requires biophysical wet-lab characterization.

The literature provides no published SAR data on naphthylalanine substitutions at Phe-4 of SS-31, making this fold genuinely exploratory. The Romanova et al. (2025) biomimetic CL-nanoliposome assay — which quantifies zeta potential changes and membrane rigidification reversal as direct readouts of SS-31/CL interaction — represents the ideal experimental platform for a direct 1-Nal versus 2-Nal head-to-head comparison. The Stefaniak et al. membrane displacement assay for alpha-synuclein provides a complementary functional endpoint. If 1-Nal shows superior potency in either assay, it would validate the compact stacking hypothesis; if 2-Nal outperforms, it would favor insertion depth as the dominant geometric determinant.

Two structural concerns deserve explicit acknowledgment. First, the 1-Nal ring geometry may produce steric clash with the Dmt-2 side chain rather than clean aromatic stacking, since Dmt (3,5-dimethyltyrosine) already presents a substantially substituted aromatic system adjacent to the backbone. The prediction suggests proximity is achievable, but the quality of the predicted stack — whether it is truly stabilizing or merely tolerated — cannot be assessed without explicit energy minimization or MD simulation. Second, increasing hydrophobicity at Phe-4 (whether via 1-Nal or 2-Nal) risks shifting SS-31's amphipathic balance toward nonspecific membrane association over CL-selective binding; this risk is identical for both variants and cannot be distinguished computationally.

In the context of the lab's running narrative, this fold pairs directly with Fold #11 to constitute a minimal regiochemical pair at Phe-4 — one of the cleanest possible SAR experiments on SS-31's aromatic residue, since the two variants differ only in ring vector geometry at constant molecular formula. The lab has now established that both 1-Nal and 2-Nal substitutions produce well-folded SS-31 analogs with identical

pLDDT (0.85), providing a structurally clean starting point for wet-lab differentiation. The next logical computational step would be coarse-grained molecular dynamics of both variants in a CL-rich bilayer, which is beyond current tool scope but is the natural extension of this work.

RESEARCH BRIEF

DISTILLATION №17 — SS-31 PHE-4 → 1-NAPHTHYLALANINE (1-NAL)

Verdict: PROMISING | pLDDT: 0.85 | Class: LONGEVITY

MECHANISM OF ACTION

SS-31 (elamipretide, D-Arg-Dmt-Lys-Phe-NH_2) is a mitochondria-targeted tetrapeptide whose established mechanism depends on direct, high-affinity binding to cardiolipin (CL) at the inner mitochondrial membrane (IMM). The alternating cationic–aromatic backbone architecture is the pharmacophore: D-Arg and Lys provide electrostatic attraction to CL's anionic bisphosphate headgroup, while Dmt-2 and Phe-4 mediate aromatic insertion into the hydrophobic CL-rich membrane leaflet. The SS-31/CL complex inhibits cytochrome c peroxidase activity, prevents CL peroxidation, stabilizes cristae ultrastructure, and preserves OXPHOS supercomplex assembly — enabling rapid ATP recovery after ischemic or mitochondrial stress. Chavez et al. extended this picture by demonstrating that SS-31 engages CL-associated proteins at CL-rich protein-lipid interfaces, suggesting that aromatic insertion geometry influences protein-lipid microdomain contacts, not only free CL binding.

MODIFICATION RATIONALE

This fold is the second member of an explicit regiochemical series at Phe-4, following **Fold #11** (2-Nal substitution, pLDDT 0.85, PROMISING). Both 1-Nal and 2-Nal have identical molecular formula and logP to each other, differing only in the vector of the second fused ring. In the 2-Nal system, the linear extension of the naphthyl ring projects distally, maximizing hydrophobic surface area for membrane insertion. In the 1-Nal system, the second ring folds back toward the peptide backbone, positioning it in proximity to the Dmt-2 aromatic system — creating a geometry more compatible with intramolecular aromatic stacking than with extended membrane insertion.

The scientific question being tested: does **depth-of-insertion** (favored by 2-Nal's outward projection) or **compact intramolecular aromatic stacking** (favored by

1-Nal's backbone-proximal geometry) better serve the SS-31 pharmacophore at CL-rich IMM interfaces? This is a well-precedented question in peptide SAR — opioid Dmt-Tic analogs and GnRH antagonists routinely show measurable affinity and conformational differences between 1-Nal and 2-Nal at equivalent positions. No published study has performed this comparison on SS-31.

PREDICTED PROPERTIES (WHERE SIGNAL IS MODERATE)

Parameter	Value	Context
pLDDT (backbone confidence)	**0.85**	Matches Fold #11 (2-Nal) exactly; high confidence in tetrapeptide fold
pTM	0.16	No complex modeled; reflects monomer only, not a failure signal
Predicted 1-Nal orientation	Toward backbone / Dmt-2 proximity	Consistent with intramolecular aromatic contact hypothesis
Aggregation propensity (heuristic)	0.068	Low
Stability score (heuristic)	0.57	Moderate; comparable to prior SS-31 variants
Half-life estimate (heuristic)	~30 min – 2 h	Moderate proteolytic resistance
BBB penetration (heuristic)	0.25	Low; consistent with mitochondrial (not CNS) targeting
Binding affinity change	Not modeled	Cardiolipin is a lipid — no protein complex available

Key signal: The structural prediction confirms that 1-Nal is accommodated within the SS-31 scaffold at pLDDT 0.85, and the predicted side-chain orientation is geometrically consistent with the intramolecular Dmt-2/1-Nal aromatic stack hypothesis. This is the cleanest possible result for a fold that cannot directly model lipid binding: the scaffold is intact, the proposed geometry is plausible, and the two regioisomers (Folds #11 and #17) are now an isogenic pLDDT-matched pair ready for experimental differentiation.

Where the signal is limited: Because cardiolipin is a lipid target, no docking, ipTM, or affinity module output is available. The predicted closeness of 1-Nal to the backbone raises a legitimate steric concern regarding clash with Dmt-2's 3,5-dimethyl substitution — the prediction shows proximity is achievable but cannot score the quality or stabilizing character of the predicted aromatic interaction.

Whether compact stacking translates to superior CL affinity, equivalent affinity, or reduced affinity relative to 2-Nal's insertion geometry is entirely unresolved computationally.

WHAT WOULD STRENGTHEN THIS SIGNAL

Computational next steps: - Coarse-grained or all-atom molecular dynamics of both 1-Nal and 2-Nal SS-31 variants in a CL-rich bilayer (e.g., POPE/TOCL mixed membrane) to assess differential insertion depth, membrane residence time, and CL headgroup contact frequency. This is the most information-dense single experiment to differentiate the two regioisomers computationally. - Explicit energy minimization of the predicted 1-Nal conformation with Dmt-2 to score the intramolecular aromatic interaction (π-stacking geometry, inter-ring distance, dihedral profile). - Ensemble prediction (multiple AlphaFold runs or ESMFold cross-validation) to confirm the backbone geometry is stable, not a single-run artifact.

Wet-lab experiments — ordered by mechanistic directness: 1. **Biomimetic CL-nanoliposome biophysics** (Romanova et al. 2025 assay): Measure zeta potential changes and membrane rigidification reversal for 1-Nal vs. 2-Nal vs. native SS-31. This is the most direct readout of differential CL-binding potency between the two regioisomers. 2. **Polarity-sensitive fluorescent analog insertion assay** (Birk et al. 2013 approach): Quantify membrane insertion depth and CL-binding affinity constants for both variants side-by-side. 3. **Cytochrome c peroxidase inhibition assay**: Functional readout of CL-protection efficacy — does 1-Nal preserve or improve on 2-Nal's inhibitory potency? 4. **NMR in CL-containing micelles or bicelles**: The gold standard for resolving intramolecular aromatic stack geometry and membrane insertion orientation in solution. No NMR structure of any SS-31/CL complex has been published; either variant would be a first. 5. **Proteolytic stability panel (plasma, mitochondrial matrix fractions)**: Tests the hypothesis that tighter aromatic stacking reduces conformational flexibility and improves metabolic resistance.

Cross-fold context: Together, Fold #11 (2-Nal) and Fold #17 (1-Nal) constitute a minimal, structurally clean regiochemical pair — identical pLDDT, identical molecular formula, distinct ring geometry. Synthesizing both as a matched pair for head-to-head CL-binding comparison is a low-cost, high-information wet-lab experiment that would provide the first published aromatic SAR data on SS-31's Phe-4 position.

> **Disclaimer:** All findings are in silico predictions only. pLDDT reflects structural confidence for an isolated peptide, not membrane-binding affinity or biological activity. Heuristic property estimates (aggregation, stability, half-life, BBB) are sequence-based approximations, not experimental measurements. This is not medical advice. Wet-lab

validation is required before any conclusions about therapeutic potential can be drawn.

SEQUENCES

NATIVE

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DArgDmtLysPhe
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MODIFIED

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DArgDmtLys(1Nal)
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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
- cardiolipin is a lipid target — no docking, complex pTM/ipTM, or affinity module output is available; pLDDT reflects backbone confidence only, not binding affinity
- heuristic property estimates (aggregation propensity 0.068, stability 0.57, half-life ~30 min–2 h, BBB 0.25) are sequence-based approximations only
- the predicted proximity of 1-Nal to Dmt-2 may reflect steric accommodation rather than a stabilizing aromatic interaction; energy minimization is needed to score this geometry
- no NMR or crystallographic data exists for any SS-31/CL complex in the published literature; the intramolecular aromatic stack hypothesis is structurally plausible but experimentally unvalidated
- increased hydrophobicity at Phe-4 (shared by both 1-Nal and 2-Nal variants) may shift amphipathic balance toward nonspecific membrane association — cannot be distinguished computationally

CITATIONS

1. **PMID —** (2013) — — The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin
2. **PMID —** (2020) — — Mitochondrial protein interaction landscape of SS-31
3. **PMID —** (2019) — — Mitochondrial protein interaction landscape of SS-31

4. **PMID —** (2025) — — Cadmium-cardiolipin disruption of respirasome assembly and redox balance through mitochondrial membrane rigidification
5. **PMID —** (2024) — — Therapeutic Peptide SS-31 Modulates Membrane Binding and Aggregation of Alpha-Synuclein and Restores Impaired Mitochondrial Function
6. **PMID —** (2021) — — Barth syndrome cellular models have dysregulated respiratory chain complex I and mitochondrial quality control due to abnormal cardiolipin
7. **PMID —** (2025) — — Elamipretide: A Review of Its Structure, Mechanism of Action, and Therapeutic Potential
8. **PMID —** (2024) — — New insight for SS-31 in treating diabetic cardiomyopathy: Activation of mitoGPX4 and alleviation of mitochondria-dependent ferroptosis
9. **PMID —** (2022) — — SS-31, a Mitochondria-Targeting Peptide, Ameliorates Kidney Disease
10. **PMID —** (2025) — — SS-31@Fer-1 Alleviates ferroptosis in hypoxia/reoxygenation cardiomyocytes via mitochondrial targeting

SOLANA SIGNATURE 478hoNENhwDBhQJtZZmULxfG4PBDicmvMDYpwxaHnhQ4qkUYTtySegJkFSEGjjvTceLocYBRUXXjfAt7UPSYFx7o
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